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# SPECIAL HEMATOLOGICAL TECHNICS*

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## A. L.E. PREPARATIONS FROM DEFIBRINATED BLOOD

PROCEDURE:

Place 25 ml. whole blood, obtained by venopuncture, immediately into a 125 ml. Ehrlenmeyer flask containing a dozen medium sized glass beads. Agitate constantly until defibrination of blood is complete. Decant the liquid part of the mixture into a serology tube (leaving beads with the fibrin clot in the flask). Centrifuge the tube containing the blood at about 3000 rpm for 30 min. Remove and discard the serum portion down to about 2 mm. above the buffy coat. Using a long capillary, remove the thin layer of serum, together with the buffy coat, and place in Wintrobe tube. Allow to sit at room temperature for 20 min. then centrifuge the Wintrobe tube for 30 to 40 min. Remove and discard supernatant serum down to about 2 mm. above the buffy coat, using a long capillary pipette. With same pipette, then remove the remaining serum and the buffy coat (about equal parts of each) and place on slide. There will be considerable admixture of red blood cells present. From the material on this slide make 4 to 6 smears which should cover ½ of slide and have a good fan. Dry in air and stain with Wright's stain.

### Acknowledgments

The above procedure is a modification of the method of Dr. Wm. Creger, Chief Hematologist, Stanford School of Medicine, San Francisco, California. The idea was also suggested in a lecture given by Dr. Wallerstein of U. C. Medical School, San Francisco, California; and by Dr. Eugene Clement, then Resident of Clinical Pathology at our hospital*. Mrs. Shirley Murty, M.T. (ASCP), assisted the author in developing the practical and workable procedure presented above.

## B. THE FUNCTION OF THE MEDICAL TECHNOLOGIST WITH REGARD TO BONE MARROW ASPIRATIONS.

In many hospitals, the medical technologist should be the person to arrange and schedule Bone Marrow Aspirations. It is therefore neces-

* Presented at the 25th Anniversary Convention of ASMT, Chicago, Illinois, June 1957. 3rd. SPF Award in Hematology.

sary, for her to know what is needed for the procedure: and which department furnishes the various items necessary.

**Equipment:**

The Sterile Pack (Tray) is prepared in Central Supply and contains the following:

1. Forceps
2. Gown for Doctor
3. Towels
4. Cotton applicators
5. 4" x 4" gauze squares
6. Scalpel with #21 BP blade
7. Drape for surgical area, 2 ft. square with 4 in. diameter hole in the center.
8. 3 syringes: one 2 ml., one 10 ml., one 30 ml.
9. Hypodermic Needles, #25; (1 each) ½ in. long and 1¼ in. long.
10. Turkel Needle with attachments, etc.

Gloves, extra syringes and needles; while also prepared in Central Supply may be obtained on the wards.

The Skin Tray with Merthiolate, Novocain, Alcohol, and large Band-Aids also comes from Central Supply.

The technologist must supply the following from the laboratory:

1. Clean Slides, about 2 dozen, in covered plastic slide box.
2. Tube containing 0.2 ml. anticoagulant (Sequestrin or Heparin are preferable)
3. Small jar containing 20 ml. 10% Formalin. (Zenker's Solution is preferred by some)
4. Extra Sternal Needle—sterilized and ready to use.

**ROUTINE PROCEDURE FOLLOWED IN OUR HOSPITAL**

I. The ward sends the request for a Bone Marrow Aspiration to the laboratory.

II. The technologist first checks with the ward (unless order has the necessary information) to see if the doctor on the case, wishes to have the resident in clinical pathology perform the aspiration; or whether he prefers to do it himself.

III. The technologist then calls the doctor or resident who decides on the time to schedule the procedure. If it is to be done by the former, check with him as to his glove size and any preferences in regard to the procedure.

IV. The technologist then notifies the ward, and schedules the time that the aspiration will be performed with the charge nurse; at the same time, also giving her the following list of items to be in the patient's room at the specified time:

1. Sterile Pack (Bone Marrow) from Central Supply
2. Skin Tray from Central Supply

3. Gloves of the proper size
4. Extra syringes, etc.

V. At scheduled time, the technologist reports to the patient's room, and proceeds as follows:

1. Sees that the patient is lying flat on the bed and that the gown is ready to remove.
2. Checks to be sure that everything ordered is in the room, and clears bedside working areas.
3. Arranges tray, etc., to suit the doctor; or has a nurse do so.
4. While the doctor is preparing patient and performing the aspiration, the technologist (a) lays out a group of slides, (b) removes cork from anticoagulant tube after wetting sides thoroughly, and (c) loosens top on Formalin jar.
5. When the syringe containing @ 0.2 ml. to 0.5 ml. of aspirated marrow is handed to the technologist, she immediately makes 6 to 12 smears by first quickly placing a small drop of marrow blood on the end of 6 slides which are spread promptly. The remainder in the syringe is placed in the anticoagulant tube and mixed thoroughly. The syringe is then rinsed in the Formalin (some of the granules may adhere to the walls of the syringe or to the plunger face.) Should the doctor remove more marrow material with a second syringe—place part of this in anticoagulant and part in formalin.
6. Technologist should fan slides to hasten air drying and then they should be placed in a plastic slide box on which patient's name, etc. has been written. Replace the top on Formalin jar, and thoroughly mix the anticoagulant tube by rotating, before returning to the laboratory. Note: If a nurse has not assisted be sure that all of the laboratory items are on the technologist's tray. Place all else on the tray from Central Supply and remove from the patient's bedside.

   Before leaving the ward, report to the charge nurse that the procedure is completed.

VI. Returning to the laboratory the technologist proceeds as follows:

1. "Squash" preparations are made from granules fished out of the sequesterinized marrow.
2. 1 direct smear and 1 "squash" preparation slide (when dry) are "Test Stained" using Wright's Stain for 3 min. and Buffer (pH 6.5) for 9 min. With greatly increased cellularity, the buffering time may have to be adjusted. As soon as "Test" slides are dry, they are shown to the pathologist. He then makes suggestions as to the staining, and indicates anything he would like prepared in addition, i.e., Hemosiderin Stain, Peroxidase, Giemsa, etc.

   Note: If you wish to hold smears a short time, after drying, immerse in Methyl Alcohol.

Staining of marrow slides should always be completed as soon as pos-

sible after the smears are dry. All slides, marked with patient's name and pertinent information, should be kept in a labeled box until work on them is completed—then they may be filed.

## Summary

The routine as outlined above has reduced time loss to both the laboratory and the wards, and has increased the efficiency of the Pathology Department. This routine also applies when an "Out Patient" Marrow Aspiration is ordered. The only difference is that scheduling is done with the Emergency Department where the aspiration is done.

Using our procedure, the pathologist sees the marrow slide as soon as is reasonably possible and is able to ask for special preparations or alteration of staining, thus enabling him to function more efficiently.

By following such a routine procedure, any technician in Hematology can function adequately, and therefore Bone Marrow Aspirations can be performed at any time needed. The frequent use of blood transfusions, at present, has made this service imperative.

## Acknowledgments

The above procedure has been developed from the suggestions of Dr. Eugene Clement (1955-56) and Dr. F. F. Busteed (1956-57), Residents in Clinical Pathology under Dr. Leslie F. Grams, Pathologist, of our hospital. The utilization of their ideas and coordination of all factors entering into this procedure, has been the author's responsibility. Thank you for the opportunity to present the results from the technologists' viewpoint.

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# ABSTRACTS

### SIMPLE SPECIFIC TEST FOR URINE GLUCOSE

Alfred H. Free et al. (Miles-Ames Research Laboratory, Elkhart, Indiana) Clin. Chem. 3, 163-168 (1957).

This report presents the data that proves the usefulness of Clinistix as a simple test for the identification of glucose in urine.

The reagents involved in the glucose oxidase test (Clinistix) are glucose oxidase, peroxidase, and orthololidine. They are impregnated into a strip of stiff paper and the strip is dipped into the urine specimen. One minute later the strip is observed for the appearance of a blue color which indicates the presence of glucose.

In the presence of oxygen the enzyme catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide. In the absence of oxygen the reaction of glucose and glucose oxidase may utilize certain organic compounds as hydrogen acceptors. In the glucose oxidase test the hydrogen peroxide formed reacts with orthololidine, the reaction benig catalyzed by a second enzyme—peroxidase. This reaction results in the development of a deep blue.

Data is presented on urine sugar tests of healthy persons, random hospital patients, and on urines with specific amounts of glucose added. The urine glucose was determined by using Clinitest, Benedict's qualitative, and Clinistix. On those urines that had 0.1%—0.25% glucose added; 97% positive with Clinistix, with amounts of added glucose between 0.35% to 0.55% the Clinistix was positive in 99% of the specimens. It was also shown that there is a high accuracy with negative specimens.

### TOTAL NEUTRAL 17-KETOSTEROIDS: CLINICAL METHOD FOR MEASUREMENT

Marian S. Kafka and Philip K. Bondy (Yale University, New Haven, Conn.) Clin. Chem. 3, 178-184 (1957).

The method described in this paper is a modification of Masuda and Thuleni's method, in order to permit a larger number of samples to be run at one time. Nonbreakable and less cumbersome equipment has been substituted, and the number and delicacy of manual operations has been reduced by making fewer transfers and by simplifying certain steps in the procedure. Polyethylene bottles and a mechanical shaker have been substituted for separatory funnels and manual shaking, and by developing the color and reading it at the same time in the test tube. Combining Zimmermann reagents before addition eliminates the necessity for frequently pipeting small volumes. This method permits the analysis of 10-15 urines in a single operation without sacrificing accuracy or specificity.

The Coleman Junior Spectrophotometer is used and the specimens read at a wavelength of 510 mu. The Coleman adapter must be modified so that the light beam passes through only the top (solvent) layer in the test tube. This can be accomplished by making a wooden adapter of the specifications given in the report.

# A BRIEF REPORT ON CAT SCRATCH DISEASE*

## Nonbacterial Regional Lymphadenitis

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In 1932, Dr. Lee Foshay[1] in Cincinnati, while studying tularemia, first recognized this disease in persons who had been scratched by cats or had had contact with these animals. He did not report his work. In 1951, Greer and Keefer[2] were the first in this country to report a case of "cat scratch fever." Later that same year, Daniels and MacMurray[3] in Washington, D. C. published the first of a series of reports on this disease.

Although commonly called cat scratch disease, the name accepted by the Committee on Standard Nomenclature of Diseases and Operations is "nonbacterial Regional Lymphadenitis,"[4] which is self-explanatory.

Hanger and Rose,[5] in 1945, first prepared an antigen from diluted pus aspirated from a suppurative lymph node. When injected intradermally this material produced a tuberculin-like reaction in Hanger, who was suffering from the disease and a similar reaction in Foshay's patients.

The disease is widespread, having been reported in India, Africa, Japan, Australia, New Zealand, Italy and other countries in Europe, Canada and the United States. The majority of our cases have come from the coastal regions of the United States and Canada, with Florida ranking next to the greater metropolitan area of Washington (in which we are located). It is no respecter of race, age or sex. In our studies, 70 per cent were children. There was equal division between the sexes.

The causative agent is still unknown. The cats suspected of having transmitted the disease have had negative intradermal reactions. Cultures of the pus from lesions in these cases have been negative on all the usual bacteriological media and there has been no growth when inoculated into chick embryos. Agglutination studies have yielded no information. Inoculation of a wide variety of birds and other animals, including cats, has not transmitted the disease. Since the majority of suspected cats are in good health, investigators feel that the cat is a passive agent in transmission of the disease. Complement-fixing antibodies have been demonstrated. However, Armstrong, Daniels, et al[6] have found no "convincing evidence that the disease belongs to the psittacosis-lymphagranuloma group" as some investigators have thought.

Cat scratch disease may simulate, and may be confused with, Hodgkins disease, tularemia, infections mononucleosis, tuberculous adenitis, lymphogranuloma venereum, or lymphosarcoma. The pathology of the lymph nodes follows a definite pattern, but it is not diagnostic. The

1. W. B. Daniels and T. G. MacMurray: Cat Scratch Disease: Nonbacterial Regional Lymphadenitis, Arch. Int. Med. 88: 763, 1951.
2. W. E. R. Greer and C. F. Keefer: Cat Scratch Fever; A Disease Entity, New Engl. J. Med. 244: 545-548, 1951.
3. W. B. Daniels and F. G. MacMurray: Cat Scratch Disease; Nonbacterial Regional Lymphadenitis: A Report of 60 Cases, Ann. Int. Med. 57: No. 4, Oct. 1952.
4. R. J. Plunkett and A. C. Hayden: Supplement to Standard Nomenclature of Disease and Operations, 4th ed., J.A.M.A. 155: 1429-1431, 1954.
5. W. B. Daniels and F. G. MacMurray: Cat Scratch Disease; Report of 160 Cases, J.A.M.A. 1954: 1247-1251, April 10, 1954.
6. C. Armstrong, W. B. Daniels, E. G. MacMurray and E. C. Turner: Complement fixation in Cat Scratch Disease Employing Lygranum CF as Antigen, J.A.M.A. 161: 149-150, May 12, 1956.

* Read before the Annual Convention—Chicago, Ill., 1957.

intradermal test, which I will describe later, is specific for cat scratch disease.

The nodes of inoculation are variable, but there is usually a history of a scratch or bite of a cat, or of some contact with cats. Cases have been reported in which the skin was broken by a splinter, thorn, or some such instrument, and the patient subsequently handled a cat, and developed the disease.

Two to four days after inoculation a small ulcer may develop at the site. This primary lesion may appear at the time of lymphadenopathy. Many patients have no recollection of any lesion other than the scratch, but some describe a lesion like an insect bite.

Within two to four weeks after inoculation, regional lymphadenopathy occurs. It may be marked and associated with fever and malaise. The patients may be afebrile but most run a low grade fever. As a rule the patient usually does not appear very ill but in some, the temperature may reach 104° F. Headache is common and sometimes chills occur. A rash may develop early in the disease, but this usually lasts only 48 hours. A few cases have been accompanied by encephalitis, and one case is reported to have developed thrombocytopenic purpura.[7] The sedimentation rate may be elevated and at times there may be a mild leucocytosis. There seems to be no correlation between the total white blood count, the temperature, and suppuration. The nodes may regress spontaneously or proceed to suppuration with the development of sterile pus. Some patients seem to have been helped by antibodies, but it is hard to evaluate the value of therapy in a self-limited disease.

The intradermal test is an important diagnostic aid. Antigen[8] for the test is prepared from the pus obtained aseptically from suppurative nodes by aspiration or at operation. In collecting the pus, care must be taken to exclude any patients with a history of jaundice, because there may be a possibility of transmitting the virus of infectious hepatitis.[9] The material is diluted 1:5 with sterile isotonic saline and heated to 56° C. for one hour on two consecutive days. Marthiolate may be added in a concentration of 1 to 10,000. When sterility has been proved, 0.1 ml. is injected intracutaneously in the forearm. The test is read at 48 hours. A positive test consists of a raised central papule 5 mm. in diameter surrounded by an erythematous areola 10 mm. in diameter. Occasionally, a positive test will consist of an erythematous area 1.5-6 cm. at the site of injection without a central papule. It may be noted here that the size of the reaction is unrelated to the severity of the disease. It is known that some persons are weak reactors to one antigen and strong reactors to another. It will be noted from the table that two subjects reacted negatively to one antigen and positively to another. Eight subjects were positive to several antigens, and 4 of the 8 gave a weaker reaction to one antigen and a strong reaction to the others. (See Table.)

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[7] J. P. Belber, A. E. Davis, and E. H. Epstein: Thrombocytopenic Purpura Associated with Cat Scratch Disease; Response of Cat Scratch Disease to Steroir Hormones, Arch., Int. Med. 94: 321-325, Aug. 1954.

[8] There is no commercial source of antigen.

[9] S. S. Kalter, J. E. Prier, and J. T. Prior: Recent Studies on the Diagnosis of Cat Scratch Fever, Ann. Int. Med. 42: No. 3, March 1955.

**TABLE**
**Antigens Tested**

| Case No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | † | N.T.* | N.T.* | †††† | N.T.* | N.T.* | †† | N.T.* |
| 2 | NEG. | N.T.* | †† | N.T.* | N.T.* | N.T.* | N.T.* | N.T.* |
| 3 | NEG. | N.T.* | ††† | N.T.* | N.T.* | N.T.* | N.T.* | N.T.* |
| 4 | †† | † | N.T.* | N.T.* | N.T.* | N.T.* | N.T.* | † |
| 5 | N.T.* | † | N.T.* | N.T.* | N.T.* | †† | N.T.* | N.T.* |
| 6 | N.T.* | †† | N.T.* | †††† | N.T.* | N.T.* | N.T.* | N.T.* |
| 7 | N.T.* | † | N.T.* | N.T.* | N.T.* | † | N.T.* | N.T.* |
| 8 | N.T.* | N.T.* | N.T.* | †††† | †††† | †††† | N.T.* | N.T.* |
| 9 | N.T.* | N.T.* | N.T.* | N.T.* | † | † | † | N.T.* |
| 10 | N.T.* | † | N.T.* | N.T.* | N.T.* | N.T.* | N.T.* | †††† |

* Not tested.
†erythema 1-2cm.: ††2-3 cm., †††3-4 cm., †††† 4 cm.

Each new lot of antigen must first be tested on positive reactors as well as on healthy controls, to determine the potency of the antigen. Not all antigens are sufficiently potent to give a good reaction. How long the antigen remains potent is another question. We have found that some antigens have remained potent for at least two years.

The skin test has been found to remain positive for as long as 9 years.[10] In fact, Peterman[11] in Milwaukee reports a positive test 28 years after the illness. No false positive reactions have been reported to date. Bettley and Fairburn[12] reported three patients who had kept cats some time and had a positive skin test. None of these had a history of cat scratch disease. This would indicate that there are subclinical cases.

Report of a case: a 6-year-old girl noticed a tender mass in the right axilla. Within two days she noted a "flea bite" on the right arm. She handled cats frequently and there were many scratches on both arms. Her temperature was 101° F., and her leucocyte count was 10,000. The sedimentation rate (Wintrobe) was 15 mm. in one hour. The chest x-ray was normal except for bilateral hilar adenopathy. The intradermal test with cat scratch disease antigen was strongly positive, with a central yellow papula 1 cm. in diameter surrounded by a tender erythematous area 5 cm. in diameter. When the mass was removed surgically, the center was found to be full of pus. The wound healed uneventfully. Later, the patient gave a strongly positive skin reaction to an antigen made from her own pus.

## Summary

A review of the literature on cat scratch disease, a discussion of the intradermal test, and a report of a case is given. In its classical form, the disease follows a cat scratch or bite, with development of a primary lesion, and regional lymphadenopathy which may or may not proceed to suppuration. Adenopathy persists from 2 weeks to 4 months, with accompanying low grade fever and malaise. Spontaneous recovery occurs in 2 to 4 months. Antibiotics have no effect in most cases, but in some cases they are reported to lower the temperature and to hasten the re-

[10] R. H. Kampmeier, and O. A. Couch, Jr.: Cat Scratch Disease, J.T.S.M.A. 47: No. 7, 278-283, July 1954.
[11] M. G. Peterman: Cat Scratch Disease; Report of a Case in 1925. J. Fed. St. Louis 44: No. 5, 563-564, May 1954.
[12] F. R. Bettley and E. A. Fairburn: Cat Scratch Disease: The Intradermal Test in Controls, Lancet, 520, March 14, 1953.

gression of the adenopathy. The intradermal test is a very valuable and specific diagnostic aid, and to date there are no false positive reactions reported. The microscopic pattern is suggestive, but not diagnostic, of cat scratch disease. The etiology still remains obscure.

I wish to thank Dr. Worth B. Daniels for his valuable help in the preparation of this paper.

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# MATHEMATICS FOR STUDENTS OF HISTOLOGICAL TECHNIQUE*

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There are several acceptable texts on histology and histological technique. None, however, present the exact selections of chemistry, mathematics and physics which we believe necessary for a well-rounded training in histological technique. Therefore, we prepared a booklet of mathematics for our own use. We present here a few of the more commonly used problems. No effort is made to present all the methods for solving a problem, or even the best method. We simply present those methods which our teaching experience has found best suited to high school graduates.

## Dilution of Stock Standards

1. Students readily grasp the traditional method of calculating how many cc of stock solution are necessary to make a liter of a more dilute solution. E. G. making 70% alcohol.†

$$\frac{70\%}{95\ \%} \times 1{,}000\ \text{cc} = 736.8\ \text{cc stock}$$

But, they seldom realize that this equation really solves four different problems. We use it often to keep us out of jail when the student comes to us and says, "Sister, I'm sorry, I just wasted a liter of tax-exempt alcohol." Using this equation, we make a correction.

$$\frac{\text{Strength Desired} \times \text{Quantity Desired}}{\text{Strength of Stock}} = \text{cc Stock to be used}$$

2. Suppose the student instead ofu sing the 736.8 cc calculated above poured in the whole liter of 95% alcohol. There is no change in the 70% required, or in the 95% stock. The stock to be used is known here to be 1,000 cc. What is changed, therefore is the "Quantity Desired." Therefore, we substitute "X" in that place and solve.

$$\frac{70\% \times X}{95\%} = 1{,}000\ \text{cc}$$

Solving, X = 1,357.14 cc = Total amount or Quantity Desired. It is advisable to demonstrate to the students the feasibility of such an answer. For example, 736/1000 is approximately 7/10. 1,000/1,357.14 is approximately 10/14. These two fractions are approximately proportional.

3. Suppose a technique indicates that a 2% solution is desired and calls for dilution of 4.85 cc of stock to 250 cc. But, the technique does not specify what stock solution to use, for example, glacial acetic acid or a percentage solution of acetic acid. Using this same equation again, the student can calculate his own answer. Here, the X is substituted for the "Strength of Stock" in the denominator.

* Presented at the Silver Anniversary Convention of ASMT, Chicago, Illinois, June, 1957. Third Educational and Professional, SPF Award 1957.

† Canadians use in place of our 95% alcohol, G.B. 94 pure spirit, o.p.

$$\frac{2\% \times 250 \text{ cc}}{X} = 4.85 \text{ cc stock}$$

Solving, X = 101%, obviously a high grade of glacial is required.

4. Or, suppose the text gives the technique for making a stain. But, the student wishes to know what is the percentage strength of the solution. Using this same equation, he can calculate this answer. Here, he will substitute "X" for the "Strength Desired" and solve.

$$\frac{X \times 250 \text{ ml}}{98\%} = 1.9 \text{ ml stock to be used}$$

Solving, X = 0.75%

## Hydrates and Anhydrides

It is sometimes desirable, when chemically permissible, to substitute hydrates for anhydrides and vice versa, or to substitute one hydrate for a higher hydrate. For example: How much sodium carbonate dihydrated would be used instead of 100 grams of anhydrous sodium carbonate? Our formula is:

$$\frac{\text{Gm. Anhy. Desired}}{\text{Mol. Wt. Anhydride}} = \frac{\text{Gm. Hydrate}}{\text{Mol. Wt. Hydrate}}$$

Substituting the figures into this equation, we have:

$$\frac{100 \text{ gms.}}{105.99} = \frac{X}{141.99}$$

Solving, X = 133.9 gms. Again, the student should realize that the answer is reasonable. It should require more of the di-hydrate because part of its molecular weight (2 × 18 gms) is the weight of the water of crystallization.

## Grams and Cubic Centimeters

In high school, students are commonly taught that grams and cc are interchangeable.** That is true only with regard to water, because by definition, a gram is the weight of 1 cc of water at 4° C. Now, students must begin to think of the gram as a measure of weight (gravity), and of the cc as a measure of volume (space occupied). Density is the combination of these two. It is the weight of a substance per unit volume.

$$\text{Density} = \frac{\text{Weight}}{\text{Volume}}$$

Going another step, specific gravity is the density of a liquid or a solid*** with relation to the density of water. Except in research work or in the extremes of climate, the density of water is accepted to be one (1). Hence, the density of liquids and solids is identical to the specific gravity. That is, the specific gravity is the weight of 1 cc. The specific gravity of every chemical should be included on the printed label of the bottle. Now, suppose a student wishes to make a solution of 2/3 normal sulphuric acid. How does he convert the required 32.69 grams into cc?

** 1 ml is equivalent to 1.000027 cc and are used interchangeably.
*** Specific gravity of gases and volumes are with reference to air.

Remembering that the specific gravity is the weight of 1 cc, we have:

$$\frac{\text{Wt. 1 cc (Sp. Gr.)}}{\text{Volume 1 cc}} = \frac{\text{Required Weight}}{\text{Required Volume}}$$

Substituting the data from the problem we have:

$$\frac{1.98 \text{ gms.}}{1 \text{ cc}} = \frac{32.69 \text{ gms.}}{X}$$

Solving, X = 16.51 gms. Again, the students should check the reasonableness of this answer. Since 1 cc weighs almost 2 gms. it is proportional that 16.51 cc would weigh approximately 32 gms.

### "Impure" Chemicals and Unsaturated Chemicals

Some of the chemicals used as mordants contain chemical "impurities." While these may not interfere chemically, they should be compensated for quantitatively. An analagous problem exists when a liquid is incompletely saturated with the active ingredient. For example, chlorine gas is only 37% soluble in water. Therefore, commercial hydrochloric acid is only 37% (approximately) saturated or "pure." Suppose the problem is to make a 3 molar solution of hydrochloric acid from a solution whose label reads, "36%" and specific gravity "1.194." This problem involves two solutions. First, we must find the exact amount of "pure" HCl there is in 1 cc of the unsaturated solution.

1.194 Specific gravity of the stock solution
$\times$ 0.36 percent of purity
0.42984 gms of HCl per 1 cc of stock solution

Second, we use this weight and set up a proportion:

$$\frac{\text{Wt. 1 cc "Pure" Chemical}}{1 \text{ cc}} = \frac{\text{Grams of "Pure Chemical" Required}}{\text{X cc of "Impure" Chemical}}$$

Substituting,

$$\frac{0.43 \text{ gms}}{1 \text{ cc}} = \frac{109.35 \text{ (required for 3 Molar HCl)}}{X}$$

Solving, X equals 254.3 cc of commercial hydrochloric acid required.

### Normal Solutions

The average high school graduate has very little concept of normalities. We find they grasp it best in terms of money. They already realize that it requires a greater number of small valued coins to accumulate a given sum, than it does of large valued coins. That is they understand that the number of coins is inversely proportional to their value. From this we move on to an understanding of normality. They can thus more readily see that to obtain a given amount of equivalents, it requires a larger quantity of a small normality than it does of a larger. And the equation becomes:

$$\frac{\text{Normality}}{\text{Normality}''} \text{ as } \frac{cc''}{cc}$$

Crossmultiplying, this equation becomes

$$N \times CC = N'' \times CC''$$

which is the formula many of us learned without knowing just where it came from. An example: How many cc of 0.05N NaOH are equivalent to 100 cc of 0.10 N Na OH?

$$100 \text{ cc} \times 0.10 \text{ N} = \text{X cc} \times 0.05 \text{ N}$$
$$\text{X} = 200 \text{ cc}$$

Note, in this form the two factors on the left side of the equals sign and the two factors on the right side of the equal sign are multiplied independently.

## Conclusion

While nothing new is presented, it is hoped that this review of mathematics may prove useful as a guide for others who are teaching histological technique to high school graduates.

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## IN MEMORIAM

Lillian L. Garner, Oklahoma—November, 1956
Lester C. Sigrist, Texas—November, 1956
Flonnie Godwin Fisher, North Carolina—February, 1957
Rhea Post Chandlee, New York—March, 1957
Carolyn Bomke, Florida—March, 1957
Mrs. Betty Goeppner, Illinois—1957
Alfred L. Carlson, Iowa—May, 1957
Aubrey Lewis, Minnesota—May, 1957
Sr. Mary Judith Sweeney, Minnesota—May, 1957
Jane C. Rizzo, Michigan—June, 1957
Sr. Mary Ortrude Feichtinger, Nebraska—July, 1957
Myra Effinger, Pennsylvania—September, 1957

A little while the labor,
Eternal the repose;
A little while the trials
That with this life will close;
And then unending gladness,
And unfading crown,
A day on whose calm beauty
The sun goes never down.

One night the shadows linger,
And then the morning breaks,
And God's own Hand the burden
From weary shoulders takes;
And thou shalt see His glory,
And hear His words "well done"
The strife forever over,
The battle fought and won.

—Anonymous

# SOME LEGAL PITFALLS OF A BLOOD BANK AND ITS TECHNOLOGISTS

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Since a discussion of the legal pitfalls of blood banking involves a consideration of a number of different activities, I believe it might be helpful to you if I were to indicate these activities first so that you may better appreciate the over-all picture. I wish to cover five main topics: first, legal problems not connected with the scientific activities of the blood bank; second, legal problems connected with the preservation, storage and distribution of blood; third, legal problems connected with the performance of a phlebotomy, both before doing it, while doing it and after doing it; fourth, legal problems connected with the performance of a blood transfusion; fifth, a few medicolegal loose ends.

First, then, what is the liability of a blood bank for injuries to a donor not connected with the scientific activities of the bank? This discussion can be brief. I refer to injuries caused by defects in and about the premises themselves such as falls on slippery floors, overheated radiators, broken stairs, or the like, and those caused by the use of defective equipment such as a wobbly table, broken chairs, unsafe stools to sit on, falls against misplaced articles on the floor, loose rugs. There are many, many ways in which people are hurt in and about buildings. The liability of the bank would be similar to the liability of a hospital for failing to maintain its premises and equipment in a reasonably safe condition and to protect users of the premises from what might be called hidden dangers. I merely mention these as a warning that some attention must be given to these matters even though blood banking is an activity which is basically non-profitable and which depends to a large extent on the donations and generosities of others.

The second realm of blood bank activity out of which legal complications might arise is in connection with the preservation, storage and distribution of the blood itself. Here, the legal principles applicable are somewhat akin to those which apply to the manufacturer who collects a product, packages it in sealed containers and markets it through wholesalers and retailers to the ultimate consumer-vendors of soft drinks, for example. The precautions necessary to avoid liability are pretty obvious to all of you and are no doubt applied by all of you every day. Mention of a few, however, might be helpful. (1) Recognized methods of collecting the blood, using sterile techniques, in pyrogen free containers, will give a reasonable amount of safety from contamination by bacteria and fungi. (2) Adequate controls should be provided so that the containers will not subsequently become contaminated and so that the blood will be stored at proper temperatures. (3) Checks and double checks must be utilized to be sure that all blood is correctly typed and further that the individual containers in which the blood is stored are accurately labeled. In short, the blood bank must be able to prove that the container of blood was prepared with due and reasonable care, using all the methods that are known to science to keep the blood or blood products free from

[1]Presented at the Silver Anniversary Convention of ASMT, Chicago, Illinois, June 1957.

contamination, from pyrogens and from infection by the virus of hepatitis, and that the label correctly describes the contents of the bottle. When a blood bank has used every possible precaution to prevent the transmission of disease by foreign agents, the blood bank can be said to have used due and reasonable care to prevent harm which, though it may be foreseeable, is still not preventable.

The third area of activity out of which legal responsibility might arise is connected with the performance of phlebotomies. If we start with the premise that the bank is liable for injuries traceable to its negligence, we can perhaps best grasp the over-all situation by dividing the discussion into three parts. The three divisions relate to preliminary procedures, the phlebotomy itself and post-phlebotomy procedures.

What preliminary procedures should a blood bank pursue? Parenthetically, I might say that I'm in a vulnerable position in discussing this matter. With the exception of a Texas case, which you may know about and which I'll discuss later, there are few definite guideposts. Therefore, the following remarks are purely my own personal feelings as to how I would advise a client to act in the matter. You must be judge of whether or not I err too far on the side of caution. You see, I would much rather keep a client out of trouble than defend him if he gets into trouble.

a. One of the first, if not the first, thing to do is to obtain an adequate consent from the donor. The purpose, of course, is to protect the bank from a subsequent charge of assault and battery. The application of the needle to the donor's arm is at least a technical assault and battery and an action for damages therefor could be sustained if no consent has been obtained. It is quite likely that mere submission to the procedure would be construed as consent but preferably the consent should be in writing and should be read to the donor so as to be sure he has understood what he is doing. It should be signed and retained in the bank's files until the period of limitations during which a suit may be filed has passed. In most states suits must be filed in one or two, sometimes three years, depending upon specific statutes of limitations.

b. After an adequate consent has been obtained, the bank should then make sufficient physical examination of the donor, including a detailed history of prior diseases, to ascertain that the donor is not suffering from any condition which militates against his giving blood. This examination should include measuring blood pressure and temperature, hemoglobin test (anemia), making a chest (lung) examination and doing a urinalysis (nephritis, diabetes). An examination should also be made to determine the presence of such detectable conditions as colds, sore throats, skin rashes, etc. Of course, the questioning would discover how recently the donor has given blood in the past. Basically, the bank does not want blood from sick persons, and it is protecting itself and subsequent recipients when it is selective with its donors. More than this, however, it is conceivable that a person, through an excess of generosity or patriotism or family need, may consciously or unconsciously conceal conditions relating to his own health. I believe a bank has a duty to protect a donor from things he doesn't know or doesn't wish to tell. Certainly most donors are not aware of the many circumstances

under which it would be an unwise or dangerous act for them to lose some of their blood. Even an adequate consent will not relieve a bank of liability for its own negligence and I believe a bank is negligent if it takes blood from a donor who is physically unfit to give it and who suffers injuries as a result of the donation.

What circumstances arising out of the phlebotomy itself might give rise to a law suit and what precautionary measures should be taken?

First, I would like briefly to mention the only case on the subject that I know about. A Mrs. Brown, living in San Angelo, Texas, went to the Shannon West Texas Memorial Hospital, in response to a request from the hospital, to donate some blood. The hospital was a collecting unit for the blood bank at Baylor University. She was directed to a certain room and there two nurses swabbed her arm, used a hypodermic needle to deaden the arm and then inserted a larger needle into her arm for the purpose of drawing blood. Great difficulty was encountered in inserting the needle into the vein, it took more than six minutes and was very painful. After the donation, the nurses taped gauze on Mrs. Brown's arm, gave her a band-aid and told her to put the band-aid on when she took the gauze off. She then returned home to her ranch, with her husband, which was about an hour's ride from San Angelo. She testified that she suffered from the time the needle was inserted to the time of the trial. There was no dispute but that she sustained a serious injury and had to be hospitalized for quite some time. Mrs. Brown contended that the injuries were caused by the use of a non-sterile needle. So far we have what looks like a tough case—difficulty in inserting the needle, the blood donation and serious injury immediately following the procedure. The trial judge, however, directed a verdict in favor of the hospital and the court of civil appeals of Texas affirmed such ruling. Was it because the hospital or blood bank could not be liable for such injuries? No it was not. The reason was simply that Mrs. Brown had failed to prove the source of the infection. The court said that what is an infection and where does it come from are matters which can be determined only by medical experts. Mrs. Brown's experts were able to say that the infection might have come from the needle—they were unable or unwilling to testify that it did come from the needle. The lesson to be learned from this case is that liability does exist for such injuries to a donor, assuming the proof to be sufficient. There is no reason to believe that liability doesn't also exist in the other circumstances mentioned. Don't give your donors a chance to prove this!

a. Probably the most important basic requirement, and the one which I'm sure first comes to the mind of all of you, is the exercise of the utmost care and caution in the selection of the technologists who are to handle the actual technique of the phlebotomy procedure. Under the legal doctrine known as respondent superior, the master is held to be liable to third persons for injuries caused by the servant in the normal course of her duties. Some states have held that in the case of charitable hospitals, where the injured person is a beneficiary of the charity and the hospital has exercised due care in the selection of the servant, no liability will attach to the hospital for the servant's negligent act. There is a difference between a charitable corporation and a non-profit corporation, however, and it is

difficult to see how a blood donor can be called a beneficiary of a charity. The situation would seem rather to be the reverse. Therefore, I believe a blood bank would be liable for injuries caused by the negligence of its employees and that a bank should make every effort to hire technologists who aren't likely to cause injuries to donors.

b. What are the injuries which might occur?
   1. Negligent and careless insertion of the needle in the donor's arm so that unnecessary damage is caused to the skin at the site and to surrounding tissues.
   2. Negligence which results in a needle breaking off in the donor's arm and resulting in the need for subsequent surgical removal. Perhaps the possibility of these examples is remote, but it should be guarded against.
   3. Possible air embolism caused by faulty manipulation of the needle or faulty handling of the equipment.
   4. Probably the most important and likely way in which an injury may be received is in the use of non-sterile equipment or non-sterile techniques. Even though it would seem that there could be no excuse for such injuries to arise, I believe the bank would be liable if they did. Therefore, the utmost care should be taken to prevent them.

How may injuries occur to the donor after the phlebotomy has been performed? Once the actual blood giving is over, the chances of any injury arising rapidly disappear. I believe that a small amount of duty to the donor still exists, however.

a. The puncture wound itself, small as it is, is still a likely portal of entry for germs. It should, therefore, be protected, by sterile measures, from the possibility of subsequent infection.
b. Donors should be restrained from violent activity for such time as the physician in charge deems advisable.
c. They should be cautioned as to permissable activities and as to suggested diet and instructed as to how long it will take the body to replace the blood lost.
d. They should be cautioned about donating blood again at too frequent an interval.

I will admit that the possibility of a person suffering an injury because he exercises too vigorously after a transfusion is remote. I have discussed that problem with a well-known physiologist and he says he has never heard of such a thing. However, it is true that while blood plasma will be replaced within an hour or two after a blood donation, it does take up to a week or so for the body to completely replace and restore the cells that were lost. Someday that one case might come along when a donor contracts some condition because of the deficiency of his blood structure and the injury could have been avoided by a word of advice by the bank following the donation. One example of an injury arising after the performance of a phlebotomy is furnished by Saltzer V. Reckford, a 1935 case coming out of the New England area. In that case the patient remarked that he felt faint after giving blood and the physician seated the patient in such a position that when the patient fainted he fell against a sterilizer and was scalded. He sued the physician for mal-

practice and the court denied a recovery. Suppose the facts were just a little different, however. Suppose there was no physician in attendance and the technologist failed to heed the patient's warning that he was feeling faint. A subsequent fall and injury could quite likely result in liability on the part of the blood bank.

The fourth area of medicolegal difficulties relates to the performance of a transfusion. I am assuming that the nursing functions necessary in the performance of a blood transfusion are not usually performed by members of your group, but rather by registered nurses acting under the direction and supervision of the physician in charge of the case. The blood bank technologist has a most pertinent relationship to the procedure, however, because it is the technologist who types the blood to be used, types the blood of the patient, labels the blood to be used and supervises its storage, etc. Some of these obligations were mentioned previously but let me repeat them now. I understand that repetition is an approved form of emphasis for educational purposes.

Although the blood transfusion is now generally considered to be a relatively minor procedure, it is nevertheless responsible for a significant number of medical accidents each year throughout the United States. The technique has become so routine that many physicians have a tendency to disregard the inherent dangers that accompany blood and plasma transfusions but that are not usually present in other intravenous procedures. It has been estimated that the mortality due to blood transfusions is about one death in 1,000 to 3,000 transfusions. It has also been estimated that as many as 3,500,000 transfusions are given each year in the United States. As a cause of death, the blood transfusion ranks with appendicitis or anesthesia. Yet, despite these facts, blood transfusions have been given by some physicians merely to facilitate the more rapid recuperation of a "run-down" patient, without even the suggestion of an emergency. Inasmuch as the transfusion of blood is associated with signficant medical and legal hazards, such treatment should be prescribed only in instances in which it is essential for the patient's recovery.

It would seem to go without saying that accuracy in blood grouping and cross-matching tests is fundamental to a safe blood transfusion. In giving a blood transfusion, the physician, of necessity, must frequently rely entirely upon the laboratory. If blood is incorrectly grouped or cross-matched by the laboratory, the physician has no way of correcting the mistake. Therefore, the utmost care must be taken to provide precautionary measures in the laboratory that will minimize the possibility of errors. Those in charge of such laboratories should entrust grouping and cross-matching tests only to reliable and specially trained technologists. Many of the accidents that have occurred are directly attributable to untrained interns and physicians who performed these tests at night or on holidays, in the absence of regularly assigned technologists.

Even when grouping and cross-matching tests are performed by highly skilled persons, labels are sometimes switched or bottles of blood mislabeled. A transfusion accident is practically inevitable once a bottle of blood has been mislabeled. Accidents occuring because of mislabeling seem to be just as prevalent as those resulting from errors in blood grouping or cross-matching. Besides errors in blood grouping, accidents

occur because of clerical errors or sheer carelessness, as in the case in which one of two patients with similar or identical names may require a transfusion and blood is administered to the wrong person. If a patient suffers a prolonged illness or dies as a result of a transfusion of blood of an incorrect group, the patient, or his family if he does not survive, may be entitled to damages unless the immediate need for blood to sustain life allowed no time for adequate tests. The transfusion of blood of an incompatible group is prima facie evidence of negligence and legal liability.

In order to illustrate the medicolegal responsibilities that are associated with the taking or administration of blood, a review of some of the important reported cases may be helpful.

In Necolayff v. Genesee Hospital, a New York case, the plaintiff was placed by her own physician in the defendant charitable hospital as a paying patient. While she was recovering from a surgical operation there, an intern and a nurse entered her room and told her that she was to have a blood transfusion from her daughter. Although she protested and informed them that she had no daughter, they nevertheless administered the transfusion. As a result she became seriously ill and temporarily insane. Actually, the transfusion was intended for another patient. The court held the hospital liable for damages and asserted that a charitable hospital could be held liable for an injury to a patient resulting from the negligence of an employee acting in an administrative capacity even though such employee be a physician, nurse, or other person normally considered professional. The court reasoned that giving a transfusion to the wrong patient was negligence and the entrance of the intern and nurse into the wrong room caused the professional nature of their errand to cease, thereby making the hospital responsible for such negligence.

Another New York case containing several interesting medical and legal points is Berg v. New York Society for the Relief of the Ruptured and Crippled. The plaintiff, Mrs. Berg, suffering from rheumatoid arthritis, entered the defendant's hospital for a course of treatment that included the administration of 55 cc. of blood. Prior to the performance of the transfusion, a sample of her blood was sent to the hospital's laboratory for analysis. The technician who tested it reported that Mrs. Berg's blood was type A, Rh positive. She was transfused with Rh-positive blood, but the transfusion was stopped when she started to develop an unfavorable reaction. A few months after she was discharged from the hospital she became pregnant and was directed by her family physician to a laboratory for the purpose of determining her blood type and Rh factor. It was then discovered, and later verified, that she was type A, Rh negative, and not Rh positive. During the course of Mrs. Berg's pregnancy it was established that the fetus was an Rh-positive one, since her titer index rose substantially. She was advised that this increased titer would in all probability be fatal to the fetus. Later the fetus died, but Mrs. Berg was advised to carry through for the full period. Because of the circumstances, the attending physician at delivery found it necessary to use manual pressure upon the abdomen for a much longer period than is usual or customary. This caused various internal difficulties that required medical treatment and ultimately a vaginal hysterectomy.

Suit was brought against the attending physician and the defendant hospital. The case against the physician was dismissed, but, after a trial without a jury, the court awarded a judgment for the plaintiff against the hospital on the ground that the act of the laboratory technician was an administrative and not a medical act and that the hospital was therefore responsible for the technician's negligence.

From this decision, the defendant hospital appealed to the appellate division which concluded that the negligent act in this case bore a sufficiently direct and immediate relationship to the care and treatment specified for the plaintiff by her own physician to require it to be construed as a "medical" act for which the hospital could not be held responsible. The judgment of the trial court in favor of the plantiff was, therefore, reversed.

Then the plaintiff appealed to the Court of Appeals, the court of last resort in New York. The first paragraph of its opinion clearly states the gist of the decision:

> The blood test was a "medical" act in the sense that it was preparatory to a transfusion. However, the test was performed by a technician who was employed and paid by the hospital. She was no independent practitioner of a learned profession, working in but not for the hospital. She was a salaried employee doing routine work. This particular hospital as the employer of this particular young women is liable for her negligence.

The judgment of the trial court in favor of the plaintiff and against the hospital was therefore reinstated.

Probably the most serious risk relating to blood transfusion and one that defies medical science and preventive measures is the danger of transmitting hepatitis. Careful screening and questioning of donors may to a limited degree lessen this risk. However, a healthy person who gives no history of hepatitis or jaundice and no clinical evidence of liver disease may nevertheless carry the virus of hepatitis. Dr. Wiener has estimated that this complication is a result of blood transfusion in 1 out of 500 cases. Although the development of hepatitis is recognized as one of the calculated risks of blood transfusion, the danger of this disease cannot be ignored and the hospital blood bank probably has a medical if not a legal duty at least to inquire into the medical history of a prospective donor and to reject those donors with a recent history of the disease. There are no reported court cases in which a hospital blood bank has been charged with negligence on this score, but, since such inquiry is customary, a patient injured by contaminated blood under circumstances in which it is shown that the use of such blood could have been avoided by reasonable diligence and inquiry probably would have a right to recover damages.

A few years ago, a patient who had contracted jaundice from a blood transfusion sought to hold a New York hospital liable for damages even though no claim of negligence on the part of the hospital was made. In Perlmutter v. Beth David Hospital, the plaintiff became ill with jaundice after receiving a blood transfusion while a patient at the defendant hospital. The hospital charged her $60 for this blood that the hospital had purchased from the third party defendant, the Blood Transfusion Association. The plaintiff alleged in her complaint that the blood was

responsible for her illness and that the hospital had breached implied warranties of fitness and merchantability in providing contaminated blood. The pleadings were not framed in negligence but in warranty. Section 15 of the Uniform Sales Act, which has been adopted in New York, provides that in every "sale of goods" there are implied warranties as to the quality of goods. The plaintiff contended that she "bought" blood from the defendant hospital, that she was billed separately for this purchase, and that as a result thereof she suffered personal injury that entitled her to damages. Accordingly, she claimed that there was an implied warranty that the blood furnished was suitable for a blood transfusion. The defendant, Beth David Hospital, filed a motion to dismiss the complaint in which it contended that the transaction involved "services" and that the implied warranties of quality found in the Uniform Sales Act do not apply to transactions that are not "sales of goods." The trial court denied the motion to dismiss the complaint, holding in effect that if the plaintiff could prove the allegations of her complaint she would be entitled to a verdict.

The defendant appealed and in a 4-to-3 decision the Court of Appeals reversed the trial court and dismissed the complaint. The majority held that the supply of blood by the hospital for a price was not a sale but merely an incident of an entire contract for services. The Uniform Sales Act attaches implied warranties to sales, but these warranties are not implied in contracts for services.

Notwithstanding the result in this case, there is still the very real possibility that under a similar set of facts a court may yet see fit to impose liability upon a supplier of blood without proof of negligence. Since the Perlmutter case was a 4-to-3 decision, a change in the composition of the New York Court of Appeals might produce an opposite ruling if these facts are again presented to the Court. Furthermore, courts in other jurisdictions conceivably may be more influenced by the dissenters than the majority, since the decision has been criticized by a number of legal scholars.

One conclusion is evident from the Perlmutter case: that there is the very real possibility that other courts, and even the New York court, may yet hold hospitals and blood banks to be insurers of the quality of blood they furnish. The American Medical Association Law Department has given the problem considerable thought and offers these suggestions. First, it would seem that as a precautionary measure hospitals should change their billing practice with reference to supplying blood. Instead of making a charge for blood, the hospital should make an equivalent and specific charge for the use of its facilities and the services of its technicians who assist the physician. Second, inasmuch as the Uniform Sales Act implies warranties in the sale of goods, it is suggested that this situation be covered by a contractual provision between the patient and the hospital to the effect that the hospital does not guarantee the quality of the blood furnished. This could be included in a form of consent or agreement for blood transfusion or a similar form. A contractual provision of this nature would offer no protection in a situation where there is negligence on the part of the hospital, such as knowingly taking blood from a diseased donor or improper storage of blood, because a contract to protect oneself from his own future negligence is invalid.

However, it may offer protection against liability without fault.

In concluding this discussion I would like to mention four medico-legal loose ends which also bear an important relationship to you technologists and the blood banks to which you devote so many of your waking hours. First I would like to present the observation that a blood bank, so far as its scientific activities are concerned, should, in my opinion, be under the direct supervision and control of a licensed physician. The question has been raised from time to time as to whether or not a venepuncture constitutes the practice of medicine—or more specifically, does it constitute surgery. In my opinion, a venepuncture can certainly be classed as a surgical technique or surgical procedure, but it is not surgery of such a character as to cause the bank or the technician to be guilty of practicing medicine without a license. In the first place, there is no diagnosing, treating or prescribing for any sickness of the donor. Such elements are necessary under most medical practice act definitions. In the second place, no fee is charged. At least I don't know of any place where the donor pays a fee for the privilege of donating blood. And finally, if my first suggestion has been followed, the acts of the technicians are being performed under the direction and supervision of a licensed physician. The constant presence of a supervising physician is a safe guard a blood bank should not overlook. Second, the legal liability of many of your institutions will depend in many cases upon whether or not the institution is charitable in nature. In years past, charitable hospitals and institutions were almost without exception held to be immune from liability for the negligent acts of their employees. There has been a shift in the thinking of a number of courts, however, and at the present time I believe the court decisions would show that twenty-five states would grant immunity, nineteen would deny immunity and four would be doubtful. Thus, although the weight of older authority in the United States is on the side of non-liability of charitable hospitals for the negligence of hospital employees, the trend now is definitely in the other direction. In recent years, five states have over-ruled prior decisions and now allow unconditional recovery. I realize that this is not particularly important to most of you. If any of you are on the managerial side, however, it will do no harm to know what the trend of decisions seems to be so that you will not be taken by surprise if your state joins those which now deny charitable immunity. Third, just as the preparation and preservation of adequate and complete records are of such vital importance to hospitals and practicing physicians, so too are the preparation and preservation of adequate and complete records important to blood banks. These records should probably contain at least the names of persons who donate blood, the results of the pre-phlebotomy examination, the consent to the procedure, correct blood type of the individual, reference to any pertinent observations made of the donor during the phlebotomy and any cautionary advice given after the procedure has been completed. Probably the records should also include copies of requests for blood for transfusion purposes, the type of blood requested and the type of blood furnished and the date thereof. The keeping of these records is as important to you, who do the work, as it is to the blood bank itself because, and this brings me to the final point in this discussion; **you are personally**

**liable for any injuries resulting from your own negligent acts whether your employer is liable or not!** I mention this last, not because it is the least important, but because if you remember nothing else that I have said this afternoon I hope you will remember this. Hence I leave it with you last. It has always been a cardinal principle of American Law, and of the English common law before that, that every person should be liable for the consequences of his own negligent and wrongful acts. In this respect medical technologists are no different from any other member of the general public. They too are liable for the consequences of their own acts. I have heard nurses and technicians express the opinion that they need not worry, the hospital or laboratory as the case might be would have to pay. Perhaps the hospital or laboratory will have to pay—but, in the eyes of the law, the medical technologist also is personally liable for any damages caused by his or her carelessness or negligence. That is something which so many employees of physicians, hospitals, laboratories and blood banks either do not realize or do not remember. A plaintiff will naturally sue the defendant from whom he has the best chance of collecting a judgment and hospitals with their insurance protection are usually more solvent than a medical technologist employed by the hospital or blood bank would be. The liability is there, however, even though the technologist is completely broke. If you realize that the liability is yours, not just that of the blood bank, you may be more apt to conduct yourself so as to prevent the liability from arising.

Under the pressure of speed and overwork the technologist can very easily make a mistake. Inattention must be guarded against. In a report made by the Committee on Medicolegal Problems of the American Medical Association, Dr. Alexander S. Wiener, one of the country's leading immunologists, highlighted the important part which medical technologists have in the practice of scientific medicine by saying "the increasing use of blood in the operating room for patients under anesthesia makes it necessary to emphasize one's complete dependance in such cases on the laboratory." Many times one reads about the importance in all phases of scientific activities of the human factor. The medical technologist is one of the human factors in today's practice of scientific medicine. The importance and the responsibility of the medical technologist should never be belittled.

# THE DETERMINATION OF BLOOD VOLUME AND RED CELL SURVIVAL TIME WITH SPECIAL EMPHASIS ON THE USE OF SODIUM CHROMATE[51] *

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Knowledge of the blood volume of a patient is frequently of utmost importance. While a hematocrit indicates the proportion of red cells in the circulating blood, it tells nothing of quantity present. Eisenberg[10] states that patients with cirrhosis frequently have a normal or even increased red cell mass in spite of low hematocrit values because of an increased vascular bed or hemodilution. Determination of blood volume, therefore, is important not only as a measure of actual blood loss or deficiency, as in the various anemias and following hemorrhage, but it is also useful as a guide in the control[28] of polycythemia.

It is the purpose of this paper to present three methods for estimating blood volume and to discuss briefly the advantages and disadvantages of each. An application of one of these methods to the study of red cell survival time will also be shown.

Plasma volumes have been estimated for many years by measuring the dilution of a non-toxic dye infused into the blood stream. Perhaps the most widely used of these dyes, at this time, is Evans Blue or T-1824.[13] This compound forms a loose combination with albumin and is lost from the vascular system at a sufficiently low rate during the first hour to permit accurate measurement. As first used, this method was open to the criticism that it was invalid in the presence of icterus, hemolysis, or lipemia. However, if the patient's own baseline plasma is used in the preparation of the standard as well as for the blank, interference of lipemia or icterus may be eliminated. Error due to slight hemolysis in one or more of the blood samples may be reduced by reading the optical density of the dyed plasma at a wave length of 540 m$\mu$ as well as at 620 m$\mu$ and applying this correction[13]:

$$\text{Dye}^{620}\ \text{Corrected} = \frac{\text{OD}^{620}_{\text{total}} - 0.1\ \text{OD}^{540}_{\text{total}}}{0.944}.$$

The principal advantage of the measurement of plasma volume with T-1824 is that no special equipment or unusual skill is required for the performance of this test.

A second widely used method of the determination of plasma volume involves the use of human serum albumin tagged with radioactive iodine ($I^{131}$)[12,27,11]. Like T-1824, its precision depends on the care with which the standard is prepared and the accuracy with which its volume and the activity of the injected tracer and samples are measured. Since the standard deviation, or counting error, is approximately equal to the square root of the count, the greater the count the smaller the percentage error becomes. It is therefore advisable to have sufficient activity for the sample to yield several thousand counts in 3 to 5 minutes. One disadvantage in the use of any radioactive isotope is the expense of procur-

*Presented at the Tenth Annual Session of the Missouri Society of Medical Technologists, April 1957.

ing and maintaining the necessary counters; authorization to the physician from the Atomic Energy Commission is also required for the procurement of the isotope. Although there is potential danger in the handling of any radioactive substance, appropriate precautions taken by the technician minimize this danger. Because of the small dosage of radioiodinated serum albumin required for a diagnostic test (3-10 uc), even repeated determinations during the course of prolonged fluid therapy offer little hazard to the patient. However, there is a remote but possible danger to the patient of the occurrence of an allergic reaction if the interval between the administrations of the radioiodinated serum albumin is as long as 2 or 3 weeks. $I^{131}$ has a half life of 8 days and, upon decay, is transformed into the element telurium[131]. The possibility of a negative error of 5 to 20 per cent was pointed out by Franks and Reeve[14] who reported the tendency of $I^{131}$ labeled albumin to be adsorbed on the glass walls of syringes, pipettes, and volumetric containers; this error can be prevented by the presence of carrier albumin.

Most investigators report close agreement between plasma volumes determined by $I^{131}$ tagged albumin and T-1824. Both tracers have been shown to be equally distributed in the plasma and to disappear from the circulation at approximately equal rates. Sear, Allen and Gregerson[21] found that an average of 10 per cent of $I^{131}$ and 9 per cent of T-1824 disappears during the first hour while Schoenberger, Kroll, Sakamoto and Kark[22], in studying the permeability factor in ascites and edema, state that $I^{131}$ tagged albumin disappears at the rate of 3.9 to 4.8 per cent per hour.

Inkley, Brooks, and Krieger[16], after repeated estimations using $I^{131}$ and T-1824 simultaneously, devised an equation for predicting plasma volume:

$$PV = 668 \text{ ml.} + (30 \times \text{wt. in Kg.}).$$

Blood volume may be calculated from plasma volume by dividing the plasma volume by the plasmacrit,* or 100 — (98 per cent × Hematocrit).

Another approach to the problem of measuring blood volume is the determination of red cell mass. As early as 1919 Ashby[2] published the differential agglutination method which could be used for this measurement. In more recent years radioactive potassium,[3] phosphorus,[3] iron,[9] zinc,[9] carbon,[13] and chromium[13,14,15] have been studied. Of these, $Cr^{51}$ as sodium chromate is most widely used. Gray and Sterling[15] were among the first to explore this avenue. They reported that the $Cr^{51}$ is very firmly bound to red blood cells, apparently to the globin portion of the hemoglobin molecule. The point of attachment has been open to some question. Smolick[23] *et al* state that it is bound to the heme. All agree that the bond is a very stable one.

The technique of the determination of red cell mass has undergone many modifications. Some investigators[23] tag group O Rh negative erythrocytes but most use the patient's own cells. Some investigators[15,18] separate the cells from the plasma aseptically, tag and wash the cells and resuspend them in the original plasma for infusion, while others[19] use ascorbic acid to reduce the uncombined sodium chromate to prevent any further reaction after injection into the blood stream.

* Plasmacrit is a term used by Reeve[20] and associates to designate the percentage of plasma in blood.

The procedure as developed in our laboratory is as follows: Radioactive sodium chromate is usually obtained as 1 mc. contained in approximately 1 ml. of solution. This is diluted aseptically to a concentration of 100 $\mu$c/ml. with sterile saline and stored in a sterile rubber stoppered vaccine bottle. A decay curve is prepared on single cycle semilogarithmic paper. Since the half life of chromium$^{51}$ is 26.5 days, a line drawn from the upper left hand corner intercepts the .5 line at 26.5 on the horizontal axis. The radioactivity per ml. on a given day may be found very easily from this chart. A 100 $\mu$c dose gives a convenient number of counts in the samples. Since it is the gamma ray emissions which are determined, a well type scintillation counter is used.

I. Preparation of $Cr^{51}$ Tagged Cells.

A. Add about 100 $\mu$c sodium chromate to the 10 ml. sterile ACD solution in a special bottle available from Abbott Laboratories.

B. Withdraw 30 to 35 ml. of patient's blood and add to the ACD-$Cr^{51}$ solution. If the patient has been previously tested or treated with a radioactive isotope which remains in the blood, a blank must be prepared from a portion of this baseline blood.

C. Incubate this mixture for at least 30 minutes at 30-37° C. with occasional gentle mixing.

D. Add 50 mg. ascorbic acid to reduce any uncombined sodium chromate.

II. Performance of Test

A. Inject intravenously exactly 30 ml. of the $Cr^{51}$ tagged suspension of cells into the patient with a siliconized syringe.

B. After a mixing period of 30 minutes, remove 4-5 ml. of blood and heparinize.

1. Determine the hematocrit in a Wintrobe tube by centrifuging 30 minutes at 3000 rpm at 15 cm. radius.
2. Centrifuge exactly 2 ml. of blood and discard the plasma.
3. Count the cells for $Cr^{51}$ activity.

C. Preparation of Standard

1. Dilute 1 ml. of the original tagged suspension to 100 ml. with normal saline.
2. Centrifuge 3 ml. of the diluted suspension in duplicate and discard the supernatant solution.
3. Resuspend the red cells in about 1 ml. of saline to simulate approximately 1 ml. of red cells.
4. Count the radioactivity of $Cr^{51}$.

D. Calculation

$$\text{Total blood volume} = \frac{1000 \times \text{net}^* \text{ standard cpm}}{\text{net sample cpm/ml. blood}}$$

Red Cell Mass = Total blood volume x .98 x hematocrit.

If a blood volume has been previously determined by this method, or if the patient has received any radioactive isotope, it is necessary to subtract a blank cpm/ml. from the sample count.

The accuracy of blood volume determination with Rachromate has

* The "net" count per minute represents the difference between the count obtained and the background count.

been examined by a number of investigators. Sterling and Gray[25] estimated the blood volume of subjects, withdrew a measured volume from some, transfused others, and repeated the estimations. Their results agreed with the theoretical values within 5 per cent.

A possible source of error in red cell mass calculations lies in the hematocrit determination. The trapping of some plasma in the red cell column is conceded by everyone but there is a wide difference of opinion in the size of the correction, if any, that should be made. Chaplin and Mollison[4] found that the correction should be 4.35 per cent for a 30 minute centrifugation period at 2000 g, which is 3000 rpm at 15 cm. and 2.50 per cent for a 55 minute period. On the other hand, Berson and Yalow[3] found an error, if not corrected, of 8.5 per cent for 30 minutes at 500 g but 2 per cent or less at 2000 g. We use a 2 per cent correction.

A method of shortening the length of time required for determination of blood volume with tagged cells has been proposed by Smolick[23] and associates. They tagged the cells from 500 ml. of freshly drawn Group O, Rh negative blood and dispensed them into vials containing 10 ml. or approximately 100 $\mu$c doses ready to be given at time without further preparation.

Some investigators[23] found close agreement between blood volume values found by using tagged cells and hematocrit, with those found using tagged albumin and hematocrit. However, others[5] report that the former is usually less than the latter. The true value is probably represented by the sum of the plasma volume and the cell volume. Reeve, Gregerson, Allen and Sear[20] report the cell hematocrit value 8-10 per cent less than the sum, and the plasma-plasmacrit value 6-8 per cent greater than the sum.

There are several advantages in the use of Rachromate for blood volume estimation: (1) The isotope disappears so slowly from the vascular system that accurate determinations may be repeated during the next 3-5 days without further introduction of the tracer[15]. (2) $Cr^{51}$ was found not to combine* with new cells, as does iron, when it is released by the destruction of old cells. It remains in the spleen.[17] (3) The blood lost by patients with chronic gastrointestinal bleeding may be estimated by examination of the feces.[6] (4) Survival time of erythrocytes may be studied.

## Red Cell Survival Studies

The length of red cell survival time is of special interest in cases of suspected hemolytic anemia.[10, 24]

Ashby[2], using differential agglutination, found that the average life span of a red cell in a normal individual is approximately 120 days. Most investigators[7,8] using Rachromate found similar values which ranged from 93 days for washed cells to 127 days for unwashed cells. The technique followed in our laboratory is as follows:

A. The specimen of blood drawn 30 minutes after the infusion of tagged cells is used as the standard. It is corked and saved.

B. Withdraw a 4-5 ml. sample of blood at 3-4 day intervals for 4-5 weeks.

C. Determine the hematocrit and count the activity of the $Cr^{51}$ tagged cells remaining in 2 ml. of blood.

* *Rachromate*—a booklet copyrighted by Abbott Laboratories in 1955.

D. On semi-logarithmic paper plot the fraction of $Cr^{51}$ labeled cells remaining in the blood on the ordinate against the time in days on the abscissa according to the formula:

$$\text{Fraction of } Cr^{51} \text{ labeled cells remaining} = \frac{\text{net cpm/ml RBC in sample}}{\text{net cpm/ml RBC in standard}}$$

The $Cr^{51}$ activity may be counted on each specimen as it is taken or the specimens may be saved and counted at any convenient time. It is necessary, however, to count the standard each time a specimen is counted. This eliminates making a correction of the decay of the isotope and correlates any variation in the counting instrument due to fluctuation of line voltage or of background.

In studying abnormal or suspected abnormal patients, determination of half life is found sufficient. The half life is that point at which half the radioactivity has disappeared. For normal individuals the range reported in the literature[17, 19, 26] is from 23 to 41 days. This value is much less than half the average life span of the red cell because the disappearance curve is an exponential one, that is, the disappearance of activity each day is a fixed percentage of that remaining. Hemolytic anemias[24], especially sickle cell anemia, may cause a half life as short as 3 days. In a small series of twelve cirrhotics studied in our laboratory,[1] six were found to have shortened apparent red cell half life. There was a significant correlation between this finding and the presence of esophageal varices, serum bilirubin levels above 1.8 mg. per cent and elevated mean corpuscular hemoglobin.

In summary it has been pointed out:

(1) That Blood Plasma Volume may be determined by measuring the dilution of tracer doses of T-1824 dye of $I^{131}$ tagged human serum albumin.
(2) That Blood Volume may be calculated from the plasma volume and the hematocrit.
(3) That Blood Volume may be estimated by measuring the dilution of red cells tagged with a radioisotope such as $Cr^{51}$.
(4) That Red Cell Mass may be calculated from the blood volume so determined and the hematocrit.
(5) That Red Cell Survival Time may be determined by serial examinations of $Cr^{51}$ tagged red cells.
(6) Methods for making these determinations have been presented.

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# METHODS USED IN TEACHING HEMATOLOGY*

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Soon the minimum requirements for M. T. (ASCP) certification will require three years of college plus the twelve months training in an approved hospital. Those schools which will have to alter their present program will be adding formal college courses in biochemistry, bacteriology, parasitology, hematology, etc. With this in mind and also to be of help to those medical technologists who train students in hematology, I am submitting an outline of the methods used in teaching hematology at Wayne State University and Receiving Hospital in Detroit, Michigan.

Since its inception in 1940 we have had a four year program leading to a degree of Bachelor of Science in Medical Technology. The first two years are spent on the main campus where a counselor supervises the program to see that all the Registry required subjects are taken.

The junior year is given at the WSU College of Medicine and the various "ologies" are covered including three courses in biochemistry. The professorial staff and the necessary equipment are best found in a college of medicine.

In the winter quarter, we have a course in CLINICAL MICROSCOPY, during which hematological methods are taught and practiced. The thirteen four-hour sessions are opened with lectures *and* demonstrations of technics followed by practice by the students. The subjects covered are:

**Table I**

1. Enumeration of RBC and WBC.
2. Estimation of hemoglobin by various methods.
3. Intravenous technic. Hematocrit. Absolute values. Indices.
4. Mean cell diameter. Preparation of blood films. Staining. Differential counts.
5. Reticulocyte counts.
6. Bleeding time. Clotting time. Clot retraction time. Platelet counts.
7. Prothrombin time determination. Erythrocyte sedimentation rate.
8. Osmotic fragility. Sickle cell preparations.
9. Total eosinophil counts. LE preparations.
10. Demonstration of taking a complete blood study. Performance of same.
11. Counts and differentials on special bloods provided by the instructor.
12. Review with emphasis on absolute values and their correlation with the appearance of the erythrocytes on blood films. Loan set slides for differentials.
13. Examinations.

Throughout these periods the students are required to report the data on regular forms which are collected and tabulated. Admittedly they have acquired little proficiency or accuracy, yet they have learned theory and methodology and are far more ready to practice these during

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the two months they spend in hematology during their senior year at an "approved hospital."

In the spring quarter we give a course in **Hematology** with emphasis on morphology. There are weekly four hour sessions with a 1-2 hour lecture. These are the subjects covered in brief detail:

**Table II**

1. Composition of blood. Erythrocyte morphology and terminology.
2. Leukocytes. Normal appearance and toxic alterations.
3. Embryological development. The normoblastic series.
4. The megaloblastic series.
5. Development of the granulocyte series from reticulo-endothelium to mature forms. The myeloblast and para-myeloblasts.
6. Monocytes. Megakaryocyte series and thrombocytes.
7. Lymphocytes. Theories of blood development.
8. The anemias. Their classification. Types of RBC regeneration. Anemias due to blood loss. Hemolytic anemias.
9. Anemias due to decreased production of erythrocytes with special emphasis on pernicious anemia and the P.A. neutrophil. Anemias due to lack of hemoglobinogenic substances. Idiopathic anemias.
10. The leukocytoses and leukopenias.
11. Polycythemia. Infectious mononucleosis.
12. The leukemias and lymphomas.

We have colored illustrations made on heavy cardboard 30 x 20 inches in size that can be hung on the wall as we lecture and describe morphological details of the various cells. For every lecture we set up microscopic demonstrations and these are accompanied by typed cell descriptions. Sixteen microscopes are available.

This is somewhat time-consuming but definitely worthwhile. Representative slides have been collected throughout the years, some with marked fields that can be found with ease. The students prefer these to lantern slides, which we also use, as they say they get better morphological detail by being able to focus up and down with the fine adjustment. We stress early in the course what to look for in identifying a cell and to note the following items:

**Table III**

1. Size of cell.
2. Ratio of nucleus to cytoplasm.
3. Nuclear pattern. Internal structure of the chromatin masses versus the lighter staining parachromatin.
4. Shape and location of the nucleus.
5. The nuclear membrane. Absent, delicate or heavy?
6. Absence or presence of nucleoli.
7. The staining reaction and structure of the cytoplasm. The hyaloplasm.
8. The presence or absence of granules in the cytoplasm.
9. The type, size and distribution of the granules.
10. Pathological variations, such as asynchronism, presence of Doehle bodies, Auer rods, etc.

We give two examinations during this course, a mid-semester and a

final, and at each we give a written and a practical. For the latter we have cells to identify at the microscopes. A favorite examination question on the written is "List ten features used to identify a cell in one column and in an adjacent one apply these to a description of the P.A. neutrophil, a monocyte or some other leukocyte."

We have a loan set for each student and a syllabus with brief case histories and blood findings to accompany the slides. We aim to have examples of all the various blood dyscrasias. The students are given the numbers for the slides covered by the lecture subject and in many instances have to turn in differential counts. One or more of us are in constant attendance to assist in cell identification.

During the senior year, we start our students on collecting and performing counts on the "psycho" wards where the diagnosis most common is "mental observation" and the data mostly in the normal range. They also make up all solutions used in hematology at least once so that they review the contents of each. We give them practice on the pediatric wards and gradually work them into doing complete blood studies on patients who are diagnostic problems.

We have a daily lecture schedule for our senior students devised to include some medical application of the results of the various tests that are performed in the laboratory. We find that these stimulate them to greater interest and accuracy. These lectures are given by the pathology residents as a part of *their* training in clinical pathology. You really learn a subject when you have to teach it. The subjects covered in hematology this year were:

**Table IV**

1. **Hemorrhagic Disorders.**
   - Description of normal hemostatic mechanisms.
   - Classification of hemorrhagic disorders.
   - The vascular purpuras.
   - Bleeding time.
   - Capillary fragility.
   - Demonstration of tourniquet test.
2. **Hemorrhagic Disorders.**
   - The thrombocytopenic purpuras.
   - Platelets and platelet enumeration.
   - Demonstration of direct counts by phase microscopy.
3. **Hemorrhagic Disorders.**
   - Laboratory methods for study of coagulation defects.
   - Clotting time and clot retraction.
   - Prothrombin time and prothrombin consumption.
   - Dicumarol and heparin therapy.
4. **Hemorrhagic Disorders.**
   - Hemophilia and related disorders (PTC, etc.)
   - Circulating anticoagulants.
   - Miscellaneous (liver disease, fibrinogenopenia, etc.)
5. **Hematocrit.**
   - Macro and micro methods.
   - Corpuscular indices.
   - Absolute values.

Correlation of absolute values with appearances on stained films
Halometer and Price-Jones curves.

6. **Reticulocyte Counts and Sedimentation Rates.**
   Various methods for performing reticulocyte counts.
   Value as a laboratory procedure.
   Errors affecting the sedimentation rate.
   Value of the test.
   Pathological processes affecting the ESR.
7. **Demonstration of Bone Marrow Aspiration and Complete Blood Study.**
8. **Preparation of Material From Hematopoietic Organs for Morphological Studies.**
   Paper by Lawrence Berman, M. D. and Elsa S. Kumke, B. S., M.T. (ASCP) at ASMT convention in 1953, including 3 lantern slides.
9. **Development of Blood Cells From the Fetal Yolk Sac to Adult Life**
10. **Preparation and Value of Bone Marrow Section Material.**
    Demonstration of methods.
    Microscopic demonstration of sections.
11. **Bone Marrow Differentials.**
    Normal values (mimeographed copies).
    The M:E ratio.
    The L:E ratio.
    Megakaryocyte enumerations.
    Bone marrow patterns.
12. **Megaloblastic Anemia.**
    Including a history of P.A. from discovery of liver principle to treatment at the present time.
    Formula for the expected reticulocyte response according to the severity of the anemia.
13. **Lupus Erythematosus.**
    Discussion of the disease.
    Laboratory methods of diagnosis.
    Our method of numerical counting of:
    1. Free cloudy nuclei.
    2. "L. E." cells.
    3. Clusters.
14. **Lipoil Storage Disturbances.**
    Gaucher's Disease.
    Niemann-Pick.
    Hand-Schuller-Christian.
15. **Myelophthisic Anemias.**
16. **Hemolysis.**
    Osmotic fragility including sterile technic of Ham.
    Acid fragility.
    Mechanical fragility.
17. **Hemolytic Anemias With a General Medical Discussion on:**
    Bacterial infections.
    Parasitic infestations.
    Chemical poisons.
    Paroxysmal hemoglobinuria.

Congenital hemolytic spherocytosis.
Acute idiopathic anemia (Lederer's).
Sickle cell anemia.
Erythroblastosis—Rh factor.

18. **Hemolytic Anemias.**
Discussion from the laboratory standpoint evaluating the various laboratory tests used for diagnosis.

19. **Hemolytic Index.**
Total fecal urobilinogen excretion.
Blood volume.
Formula.

20. **Hemoglobin Electrophoresis.**
Procedures.
Interpretations.

21. **Leukemias.**
Classification of acute, subacute and chronic.
Classification according to cell types, granulocytic, monocytic (Naegeli and Schilling).

22. **Lymphocytic Leukemia.**
The classification of the lymphomas according to Lawrence Berman, M.D.

23. **Leukocytic Functions.**

24. **Differential Diagnosis of Anemias Which Present Peripheral Leukoerythroblastic Reactions.**
Dorothy Sundberg, Ph.D., M.D., as presented at ASMT convention in 1955.

25. **Special Stains.**
Mimeographed copies of directions.
Microscopic demonstrations:
Siderocytes for iron.
Le Pehne's for hemoglobin.
Alkaline phosphatase.
Peroxidase.
Giemsa.
Schleicher's decolorizer.

26. **Microscopic Demonstration of Unusual Cells such as:**
Osteoblast.
Osteoclast.
Tissue mast cell.
Sternberg-Reed cell.
Gaucher's cell.
Fat cells.
Tumor cells.
Myeloma, etc.

Using these methods, we find that our graduating students have acquired a working efficiency, a general knowledge of the scope of hematology and a genuine interest in the subject.

If an outline such as this proves of value in this subject, perhaps medical technologists specializing in other fields would contribute similar ones covering the other subjects in which students of medical technology must be trained.

# BCG VACCINE*

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The discovery of BCG was a fortuitous one. It appears that in the Pasteur Institute of Lille, there was a virulent culture of bovine tubercle bacilli isolated by Dr. Nocard of Alfort from a tuberculous mastitis in a heifer; 1/10,000 of a mg. of this culture killed a guinea pig weighing 400 grams in 60 days. This strain was used commonly in the experimental work at the Institute. In 1908 it was noted that in order to facilitate a finely divided emulsion of these organisms a drop of bile added to the agate mortar in the grinding would facilitate a better emulsion to be used for experimental purposes. It appeared that the beef bile acted like a soap, wetting the fatty surface of the bacilli and made it possible to dissociate the aggregates into their division of almost singe units. It was then reasoned that if bile facilitated the emulsification of the organisms, would it be possible to grow this organism on bile so that a finer suspension could be had at will. After some experimentation it was found that pieces of potato cooked at 70° in beef bile containing 5% glycerine and kept impregnated with the same fluid media would allow this organism to grow on the surface of the potato. Surely enough after a few months of culture and subculturing the characteristic of the organism changed from granular dry and rough to become viscous, moist and smooth. Such organism suspended easily and an emulsion was made that contained few aggregates or clumps. Assaying the virulence of these cultures it was found that after the first few transfers there was an increase in virulence, but gradually after repeated subculturing there was a loss of virulence so that after 15 transfers (once every 3 weeks) 1 mg. failed to produce ill effects in guinea pigs. Finally, after 231 passages over 13 years, the organism failed to produce progressive disease in guinea pigs, rabbits, cows, etc. even when injected in large doses. This attenuated bovine tubercle bacillus now called, Bacillus, Calmette and Guerin, was now considered ready for human inoculation.[1] What had transpired during these successive transfers on bile potato? The French believed that there was mass mutation due to the presence of bile, to form this attenuated bovine tubercle bacillus. Another possibility, however, is that the bile inhibited the virulent organisms but allowed the attenuated ones to grow much the same as is suspected happens when INH is added to growing cultures of tubercle bacilli. We know now that many of the INH resistant strains are likewise less virulent for guinea pigs than the sensitive organisms.

*Cultural Characteristics*

It is not possible by colony morphology to differentiate unequivocally virulent from attenuated strains of tubercle bacilli. Middlebrook et Dubos[2] have described certain cultural characteristics which distinguish virulent from avirulent tubercle bacilli but not attenuated forms (see below). On potato sauton media which is used in the preparation of BCG vaccine, the organism grows on the potato beginning with a pinhead size granule which gradually enlarges to several mm. becomes

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fused to form single or intertwining coils after 10 to 14 days. By this time the surface of the sauton is also covered by a thin undulating veil with an occasional elevated opaque coil. In the preparation of vaccine, this veil is transferred onto synthetic fluid sauton media. The culture grows on the surface of the sauton medium as a pellicle—first as a smooth veil, then undulating, and finally the same intertwining coils as described on the potato. The fluid portion of the media remains crystal clear. The pellicle used for vaccine is 8 to 10 days old.

On oleic acid albumin agar media, 3 types of colonies may be seen. The spreading type, which has a dense opaque center surrounded by a halo consisting of serpentine strands folded close together; the intermediate type which has a dark center, much larger than the colony described above, and the halo is reduced to a small band but still retains the serpentine pattern, and the non-spreading type which has a dark center with or without a rudimentary halo. Virulent tubercle bacilli have predominantly spreading colonies whereas avirulent tubercle bacilli predominantly non-spreading ones.

*Morphology*

BCG is a slender slightly curved rod with rounded ends. The internal structure is that of dense granules suspended in a cytoplasm of lesser density. The internal masses are spherical or oval with regular or irregular outlines, sometimes two spherical polar bodies are noted especially in younger bacilli. The number and distribution of these granules is not constant, average 3 to 6. The length of the organism varies from 2 to 10 microns but the majority are between 2½ and 3½ microns. The diameter varies from .2 to 1 micron, the majority being approximately .6 micron.[3]

*Biochemical Characteristics*

BCG is acid fast as is the parent bovine strain. When stained with neutral red in alkaline solution, BCG will take up the red stain but not as intensely as do virulent strains. Avirulent strains do not discolor red. Oxidation reduction phenol dyes are recolorized by avirulent strains of tubercle bacilli, are not affected by virulent strains or by BCG.

*Preparation of Vaccine*

The standard method basically employs a surface pellicle 8 to 10 days old on sauton media for production of vaccine as described above. It has recently been proposed that vaccine be prepared from dispersed growth of organisms from the depths of a synthetic medium to which a wetting agent (Tween 80) and human serum filtrate have been added. This method is intended to eliminate the mechanical trauma to the organisms which result from grinding in the standard method. The latter method which seems attractive, however, is still in the experimental stage since the use of serum albumin might implicate virus from infectious hepatitis and furthermore the yield by this method is relatively low.

The pellicle is ground in a ball mill with steel balls for about 10 to 12 minutes at 30 rpm. For fresh vaccine preparation sauton diluted ¼ is added to make the proper concentration as determined by either Hopkins tube or nephelometry. In this country the majority of the vaccine used is freeze-dried. The choice of an adjuvant that will protect organ-

isms during the processing is critical. We have found that 15% lactose plus buffered salts, plus asparagine and glycerine is suitable. This adjuvant is added to the ball mill with the ground organisms, shaken well and then is dispensed into 5 ml. ampules placing 1 ml. in each ampule. The vaccine is then quickly frozen at a temperature of —45° C and placed in an evacuation chamber where a vacuum is obtained of about 30 to 40 microns. The sublimation is allowed to continue for about 15 to 18 hours. After this time, most of the moisture has been removed but in order to rid of the residual moisture, the vaccine is heated by means of infrared for 2 to 3 hours. After drying the chamber vacuum is broken by dry nitrogen. The ampules are sealed with heat and kept at 4° C until used.

*Standardization of BCG*[2,3]

The great advantage of the freeze-dried vaccine is that all tests for standardization and safety can be completed before the vaccine is released. Thus, viability, virulence, potency in guinea pigs and in humans can all be determined before any batch of vaccine is released for general distribution.

1. Viability: Vaccine (½ ml.) is diluted serially in log dilutions in Youmans fluid media from $10^{-1}$ to $10^{-10}$. The growth that occurs in the highest dilution is determined. Normally, growth in the standard vaccine will occur in $10^{-9}$ dilution. This would mean that there is at least 100 million organisms in ½ ml. of vaccine.

2. Virulence: This is determined by inoculating 10 mg. of BCG intraperitoneally into guinea pigs. The animals are sacrificed at intervals of 6 weeks, 3 months and 6 months. After 6 weeks there is usually appreciable pathology in the omentum consisting of large nodules up to 8 or 10 mm. in diameter. There may be small nodules on the surface of the spleen and liver. At 3 and 6 months, the nodules in the omentum have receded to measure a few mm. At no time is gross pathology noted outside of the abdominal cavity.

3. Potency:

(a) Tuberculinogenic: One puncture by a sewing type needle is made through a drop of vaccine into the guinea pig skin. After 2 weeks the animals will react to 1:100 and 1:10 of Old Tuberculin. In humans, newborns are vaccinated with the multiple puncture disc which contains 36 prongs. Tested 2 months later, all should react to 1 mg. and 90% or more to .1 mg. O.T. The U.S.P.H.S. requires 80% to react.

(b) Immunizing: Guinea pigs are vaccinated by the same dose and method as used in human beings. After 6 to 8 weeks when the tuberculin reaction is well established, an infecting dose of approximately .002 mg. or 1300 viable virulent human tubercle bacilli ($H_{37}Rv$) are introduced intraperitoneally. When 60% of the unvaccinated controls are dead the remaining animals are sacrificed. At this time there should be 80% or more of the vaccinated animals still alive. The extent and the size of the lesions of the animals should be much smaller than those of the controls. Mice are also used for immunizing potency. For this purpose 1 mg. of BCG is inoculated intraperitoneally in mice weighing about 20 grams. After 30 days, they are inoculated intravenously with

1 mg. of $H_{37}Rv$. Normally, the control animals will live approximately on the average of 13 days. In the vaccinated group as many as 70% will live beyond 30 days (method of Youmans).

4. Miscellaneous: It has been suggested that the degree of spreading colonies or cording found in any strain of BCG will be directly related to the potency of the vaccine, both from a tuberculinogenic as well as an immunologic capacity. A determination of the extent and nature of the cording is made morphologically after growing on oleic acid agar medium. The majority of the colonies should be of the intermediate spreading type. Cording can also be determined by staining growth from fluid medium. The extent to which the bacilli arrange themselves parallelly to form serpentine cords is noted. This type of arrangement should be the rule.

*Administration of BCG Vaccine*[3]

There are 3 main ways of administering BCG, orally, intradermally and, transcutaneously. The oral method was first used by the French but soon given up because the rate of tuberculin conversion was low. Recently, however, the Brazilians have reestablished this method but use larger doses of vaccine. They administer the vaccine once with a dose of 100 to 200 mg. routinely and every month for 6 months in doses of 100 mg. for a total of 600 mg. in contact cases. A high percentage of conversion and a high degree of immunity is claimed. The disadvantage of this method is that large doses of the vaccine are necessary and also that under certain conditions it has to be given over extended periods of time. The intradermal method is the one used in the Joint Enterprise-World Health Organization studies. This is a highly effective method wherein from .04 to .1 mg. of BCG is given intradermally as superficially as possible in 0.1 ml of fluid. This method has advantages over the oral in that the tuberculin conversion is more rapid, it lasts for longer periods of time and only one dose is necessary. However, complications do arise by this method, especially in newborns. It has been found in one study that as much as 20% of newborns under 1 year of age will develop draining sinuses from the local lymph nodes. This figure has varied all the way from 1 to 23%. In children as well as in young adults, the number of complications is less. However, the type of lesion is similar to that produced by a smallpox vaccination; a crusted lesion which opens and closes for periods up to 2 to 3 months and sometimes longer and always leaves a scar. Transcutaneous method: The multiple puncture method, its principle being to vaccinate at numerous sites with small doses, has to its advantage that practically no complications follow. This is true even in vaccinating newborns 2 days of age. Multiple punctures can be made by a sewing type needle held tangentially to the skin and pressed downward and then upward to engage the skin. Thirty punctures are made 4 mm. apart in 3 rows of ten. A disc with 36 prongs placed 4 mm. apart has been devised so that the punctures can be given with one stroke. The disc is held by a magnet, which serves as the holder. A separate disc is used for each inoculation. A modification of the multiple puncture method was introduced by the French where simply linear cuts are made through vaccine.

In newborns the intradermal and multiple puncture method yield

about the same percentage of tuberculin conversion. In the young adults the percentage of tuberculin conversion may be higher with the intradermal method depending upon the dose.

For the percutaneous method the tuberculin reaction becomes positive 6 to 8 weeks following vaccination. Depending upon the age the reaction will remain positive in infants for 5 to 6 years and for young adults 2 to 3 years. At this time approximately 70 to 80% will react to tuberculin (2nd strength PPD or O.T.—100 TU).

*Safety of BCG Vaccine in Man*

Except for the so-called Lubeck disaster, which occurred in Lubeck, Germany, in 1930, no serious complications following BCG vaccination were reported until about a year ago. In the 1930 episode, 251 infants were vaccinated by mouth with a culture of supposedly BCG, in which 72 died of extensive tuberculosis. The German Government placed Ludvig Lange of the Robert Koch Institute of Berlin in charge of a thorough investigation of the epidemic. After 5 years of study a volume was published by the German Government in 1935 in which it was clearly shown that the BCG vaccine used was by negligence contaminated with a virulent strain of tubercle bacilli. Because of the gross negligence shown by the workers in Germany, they were imprisoned.[3]

Recently, 1955, 4 cases of supposedly progressive tuberculosis and death developed in 3 children and 1 young adult who had been vaccinated with BCG. In all cases tubercle bacilli were isolated which did not produce progressive disease in guinea pigs. This may have been BCG or some atypical tubercle bacillus. One of the authors who reported a death, stated after making exhaustive bacteriologic studies that "at the present stage of research, we cannot prove that this is the case," that is that the bacilli isolated from the patient was derived from the ones used in BCG vaccination. Fortunately, the various bacteriologic techniques and biochemical studies cannot unequivocally differentiate BCG, atypical and virulent tubercle bacilli. Considering that a large proportion of the younger population in the Scandinavian countries have been vaccinated with BCG, and that atypical tubercle bacilli causing active disease although rare have been reported in Scandinavia and elsewhere, the possibility of the two conditions occurring simultaneously cannot be excluded. Even if one accepted the deaths in the above 4 cases as a result of BCG, considering the millions of vaccinations in Scandinavian countries alone without untoward effects, the Scandinavian state editorially that there is no reason to change the practice with regard to BCG vaccination. Other lesions have been described following vaccination, such as lupus vulgaris, ocular lesions and an enlargement of the hilus lymph nodes. These lesions have not been very common, and they have been benign in character. The most common complications following BCG are the local reactions at the site of inoculation. As already mentioned, the intradermal method especially in newborns may produce abscesses and suppuration of the regional lymph nodes. These complications are rare in older children and adults if the technique is done properly. Following the multiple puncture, complications are practically eliminated. It is of interest to note that following the oral administration of the vaccine complications

may occur. In Holland recently they reported that 4.5% of infants orally vaccinated developed cervical adenitis. However, no such complications have been reported from South America, especially Brazil.[3]

*Efficacy of BCG Vaccine*

BCG has been shown to be highly effective in the prevention of primary tuberculosis and post-primary tuberculosis including pulmonary tuberculosis. Death from tuberculosis especially tuberculous meningitis and miliary tuberculosis in the first year of life has practically been eliminated following the intradermal or multiple puncture method of BCG vaccination. Dr. Wallgren[4] states that in Scandinavia there has not been a case of tuberculous meningitis in infants properly vaccinated with BCG. Reports of a similar nature have come from Denmark, United States, Canada, Great Britain, France, Ireland and Germany, and speak for a high degree of protection afforded by BCG vaccination. Children and young adults have likewise shown a high degree of protection when BCG was used as part of an over-all tuberculosis control program. Rosenthal[3,5] reports from 78 to 100% reduction in the incidence of tuberculosis in children, depending upon the other method of tuberculosis control practiced. Aronson[6] in a 20 year report on American Indians reports an 80% reduction in mortality from tuberculosis and 73% reduction in morbidity. He found 10 times as many cases of moderately advanced pulmonary tuberculosis in the non-vaccinated as compared to the vaccinated. A most significant report was the one by the Medical Research Council of Great Britain.[7] Their general plan was to include approximately 56,000 boys and girls in their final year in secondary school, so nearly all pupils were between 14½ and 15 years of age. The studies were conducted in large and heavily populated areas in North London, Birmingham and Manchester. Children known to have been in recent contact with a case of pulmonary tuberculosis (within 2 years) were excluded from the trials because they were already eligible for BCG vaccination under a plan introduced by the Ministry of Health in 1949. Each child was X-rayed and tuberculin tested with 0.1 ml. of 1:3000 O.T. (3 TU) and if they were negative this was followed by 0.1 ml. of 1:100 O.T. (100 TU). Of the negative reactors, alternate children by a very exact method were vaccinated or not. Approximately 94% of the participants were followed for 30 months. A smaller number were followed for 4 years. To avoid bias the one who assessed whether there was tuberculosis or not after this time was kept unaware of the results of any tuberculin tests and was not told whether a vaccination had been performed or not. Their study indicated that there was an annual incidence of 1.94 cases per 1,000 participants per year in the negative reactor unvaccinated group; whereas there was 0.37 per 1,000 in the vaccinated. That is, there was an 82% reduction of incidence of tuberculosis in the vaccinated as compared to the tuberculin negative non-vaccinated controls. Similar results were obtained in nursing students by Heimbeck of Oslo and in conscripts in Sweden by Dahlstrom and Difs. In the latter study some 61,474 individuals were followed, approximately half were vaccinated and half were controls. These men were about 20 years of age. Each usually served for 1 year, but some remained on emergency service for varying periods of time,

up to 7 years. There was about 3 times as much primary tuberculosis and post-primary tuberculosis in the controls as in the vaccinated. Post-primary tuberculosis included destructive pulmonary tuberculosis with cavity formation. In this country Rosenthal[3] and associates vaccinated medical students and nurses on a control basis and found that in medical students, for example, over a period of some 13 years, there was not a case of tuberculosis in the vaccinated medical students. On the other hand, there were 10 cases of tuberculosis in the non-vaccinated group. This included some tuberculin negative as well as tuberculin positive reactors and a group in which the tuberculin status was unknown. In nursing students there was a 59% reduction in the incidence of tuberculosis in the vaccinated as compared to the controls. However, in this particular study the vaccinated were allowed to work in a tuberculosis hospital whereas the controls were not.

BCG should in no way replace the well established and effective methods of tuberculosis control but should be *used as an adjunct* added to the armanentarium of tuberculosis control procedures.

In summary, it may be stated that:

1. BCG is an effective vaccine for increasing resistance to tuberculosis.
2. BCG vaccination has decreased the incidence of tuberculosis in all age groups as compared to non-vaccinated controls.
3. BCG in the freeze-dried form is well standardized for safety, viability, and potency.
4. BCG is a safe vaccine.
5. BCG should be considered as an adjunct to the proven methods of tuberculosis control.

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## CORRECTION

PANOCELL is the registered trademark of the Knickerbocker Biologicals, Inc. for a panel of group O cells. This product was mentioned in the article, "The Rarer Antibodies, Their Detection and Clinical Significance," by I. Dunsford, September-October, American Journal of Medical Technology.

# EMERGENCY TOXICOLOGY PROCEDURES FOR THE CLINICAL CHEMISTRY LABORATORY*

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Statistics show that 4% of the total fatal accidents in a recent year were caused by poisons; i.e. 2.3 deaths per 100,000 population were accidental poisonings. Probably at least ten times this number of people were involved in accidental poisonings which did not terminate fatally. Thus the care and treatment of patients suffering from poisoning is a problem which every hospital must be prepared to face. When we consider the literally enormous number and variety of toxic materials to which everyone is exposed almost daily—insecticides, drugs, household products, industrial materials—the problem of analyzing and identifying the poison in question assumes major proportions. It is, in fact, a problem that can be handled in its entirety only by a toxicologist. However, since the services of a toxicologist are in many cases not readily available, the biochemistry technologist is frequently called upon to supply what aid she can. It is the purpose of this paper to present a series of analytical procedures for the more frequently encountered poisons which can be carried out in a routine biochemistry laboratory by an experienced technologist. These include ethanol, methanol, carbon monoxide, barbiturates, salicylates, and heavy metals.

Feldstein (1) has reported a very elegant technic for the determination of volatile poisons, utilizing the standard Conway microdiffusion cell (2).

Analyses for ethanol, methanol, carbon monoxide, and cyanide can be carried out by this means, using a total of 5 ml of blood or body fluid and a relatively small percentage of the analyst's time.

Oxalated blood is the material of choice for these analyses. In each of the following procedures the Conway cell is prepared as follows: An aliquot of blood is placed in the outer compartment, and a measured volume of the absorbing solution is placed in the center compartment. The ground glass cover of the unit is smeared with silicone grease and is placed on the unit so that a small portion of the outer chamber remains uncovered. A measured volume of the appropriate liberating agent is then pipetted into the outer compartment and the cover moved into position to cover the entire unit with an air-tight seal. The unit is then gently tilted and rotated to mix the fluids in the outer compartment, and allowed to stand for the indicated time. When diffusion is complete, the cover is removed, and a portion of the absorbing solution in the center compartment is taken for analysis.

## Analysis For Ethanol

**Reagents:**

1. Saturated potassium carbonate
2. Acid-dichromate: dissolve 3.70 grams analytical reagent grade potassium dichromate in 150 ml distilled water. Carefully add 280 ml concentrated sulfuric acid, cool, and dilute to 650 ml.

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**Procedure:**

Outer compartment:
0.8 ml blood
Center compartment:
2.0 ml acid-dichromate solution
Outer compartment (liberating agent):
1.0 ml saturated potassium carbonate
Diffusion time:
3 hours at room temperature

**Quantitative Determination:**

The acid-dichromate solution is transferred from the center well with the aid of a capillary pipet and several rinsings with water to a 10 ml graduated test tube and diluted to 10 ml with water. A blank consisting of 2 ml of the acid-dichromate solution diluted to 10 ml with water is prepared at the same time. The optical densities of the solutions are determined in a spectrophotometer at 450 millimicrons against distilled water as a reference. Standards are prepared by adding known amounts of alcohol to blood and analyzing as above. The unknown is then calculated as follows:

$$(\text{density blank} - \text{density unknown}) \times F = \text{mg\% alcohol}$$

$$\text{where } F = \frac{\text{concn. of Std. (mg\%)}}{\text{d blank} - \text{d standard}}$$

The range of concentration of alcohol which can be determined using 0.8 ml of blood is from 0 to 300 mg%. Should larger amounts of alcohol be present, the amount of blood used should be decreased to 0.4 ml, and the final result multiplied by 2.

A rough estimate of the quantity of alcohol present may be made by visual inspection of the acid-dichromate. If the color remains orange, it may be presumed that there is no ethanol present. A positive test, however, may indicate the presence of methanol, acetaldehyde, formaldehyde, or isopropanol as well as ethanol. Although ethanol is by far the most common, the absence of the other substances must be demonstrated (1).

## Analysis for Methanol

**Reagents:**

1. 0.5% chromotropic acid, freshly prepared
2. Concentrated sulfuric acid
3. 5% potassium permanganate
4. Sodium bisulfite, saturated solution
5. Potassium carbonate, saturated solution
6. 10% sulfuric acid (v/v)

**Procedure:**

Outer compartment:
1.0 ml blood
Center compartment:
2.2 ml 10% sulfuric acid
Outer compartment (liberating agent):
1.0 ml saturated potassium carbonate solution
Diffusion time:
3 hours at room temperature

**Quantitative Determination:**

Pipet 1 ml of the absorbing solution into each of two test tubes graduated at 10 ml, and label these "A" and "B." To tube A add one drop of 5% potassium permanganate solution. Allow to stand 10 minutes. To both tubes add one drop of saturated sodium bisulfite. (If the permanganate is not completely decolorized, add a second drop of bisulfite to each tube.) To each tube add 0.2 ml of 0.5% chromotropic acid. Place the tubes in an ice bath, and to each add cautiously 4 ml of concentrated sulfuric acid. Mix and place in a boiling water bath for 15 minutes, cool, and dilute to 10 ml. A purple color in tube A indicates a positive methanol. If there is more than a trace of purple color in tube B (positive formaldehyde), this method is inapplicable. Read the optical densities of both tubes in a spectrophotometer at 580 millimicrons against a water blank. The optical density of tube B is subtracted from that of tube A. Compare to standards of methanol in 10% sulfuric acid treated similarly. Results must be multiplied by 2.2 (of 2.2 ml of absorbing solution only 1 ml was used in the analysis) and by 1.21 to correct for diffusion losses.

## Analysis For Carbon Monoxide

**Reagents:**

1. Palladium chloride: dissolve 0.22 grams palladium chloride in 250 ml of 0.01 N hydrochloric acid, heating if necessary.
2. 10% sulfuric acid (v/v)
3. 0.1% gum ghatti
4. 15% potassium iodide

**Procedure:**

Outer compartment:
1.0 ml blood
Center compartment:
2.0 ml palladium chloride
Outer compartment (liberating agent):
1.0 ml 10% sulfuric acid
Diffusion time:
1 hour at room temperature

**Quantitative Determination:**

If there is no change in the palladium chloride solution the test may be presumed to be negative. In the presence of carbon monoxide, a silvery film of metallic palladium will appear on the surface. With the aid of a capillary pipet, remove the unreacted palladium chloride from beneath the metallic precipitate, and transfer to a test tube. Transfer 0.1 ml of this solution to a 10 ml volumetric flask. To a second 10 ml volumetric flask add 0.1 ml of the original palladium chloride solution. To each flask add 1.0 ml of 0.1% gum ghatti solution and 1.0 ml of 15% potassium iodide. Dilute to volume and mix. Read the optical density at 500 millimicrons against distilled water as a reference.

**Calculation:**

$$(\text{density control} - \text{density unknown}) \times \frac{0.0528}{\text{density of control}} \times 420 = \text{Vol\% CO}$$

$$\frac{\text{Vol\% CO content}}{\text{Gm. Hb per 100 ml blood} \times 1.34} = \text{\% Saturation}$$

## Analysis for Cyanide

**Reagents:**

1. 10% sulfuric acid (v/v)
2. 0.1 N sodium hydroxide
3. 1 M sodium phosphate monobasic
4. 0.25% chloramine T (keep on ice)
5. Pyridine-barbituric acid reagent (3): 3 grams barbituric acid, 15 ml pyridine, and 3 ml concentrated hydrochloric acid are placed in a 50 ml volumetric flask. Mix to dissolve the barbituric acid, dilute to volume with water, and filter. This solution must be freshly prepared.

**Procedure:**

Outer compartment:
2-4 ml blood
Center compartment:
2 ml 0.1 N sodium hydroxide
Outer compartment (liberating agent):
3-4 drops of 10% sulfuric acid
Diffusion time:
3-4 hours at room temperature

**Quantitative Determination:**

Place 1.0 ml of the absorbing solution into a 10 ml graduated test tube. Into a second 10 ml graduated test tube, place 1.0 ml of 0.1 N sodium hydroxide for a blank. Into a third test tube, pipet 1.0 ml of a standard solution of cyanide, 0.2 micrograms in 1 ml of 0.1 N sodium hydroxide. To each tube add 2.0 ml of 1 M sodium acid phosphate and 1.0 ml of chloramine T solution. Mix and allow to stand for 2-3 minutes. Add 3.0 ml of the pyridine-barbituric acid reagent to each tube. Mix and allow to stand 10 minutes. The appearance of a red color is indicative of the presence of cyanide. Read the optical densities of the three tubes in a spectrophotometer at 580 millimicrons against water as a reference. The optical density of the blank is subtracted from that of the standard and the unknown.

## Analysis for Barbiturates

Barbiturates are one of the most common causes of drug intoxication. While accurate quantitative estimations are beyond the capacity of the routine chemistry laboratory, useful information may be gained from a qualitative test such as the Koppanyi test (4). Material of choice for this analysis is urine. Approximately 100 ml of urine are acidified with dilute hydrochloric acid, and the urine extracted in a separatory funnel with 100-150 ml of ether. The ether is separated, washed once with 10 ml of dilute sodium bicarbonate solution, and once with 10 ml of water. The washed ether is then evaporated to dryness over a steam bath. The dry residue is dissolved in a few ml of dry chloroform, and an aliquot of this solution is placed in a small test tube. To this are added 3-5 drops of a 1% solution of cobaltous nitrate in methanol. The solution is mixed, and 1 ml of a 5% solution of isopropyl amine in methanol is carefully layered above it. In the presence of barbiturate, a blue to purple ring will form at the interface. Mix gently. A very rough estimate of the amount of barbiturate present may be obtained from observation of the intensity of the color, although the color reaction is not quantitative.

## Analysis for Salicylates

Salicylate intoxication is occasionally found in children. Pipet 1 ml of serum, plasma, or urine into a test tube. Add 9 ml of a 0.5% solution of ferric nitrate in 0.07 N nitric acid. Prepare a blank of 1 ml of serum or urine to which 9 ml of 0.07 N nitric acid is added. A purple color with ferric nitrate is indicative of the presence of salicylates. Compare to standards of salicylate in 0.07 N nitric acid, treated similarly. Quantitative estimation may be made by visual comparison of the unknown with an appropriate series of standards (5).

## Analysis for Heavy Metals

Arsenic, antimony, mercury, and bismuth may be detected by means of the Reinsch test (6). Material of choice is urine or stomach contents. Place approximately 20 ml of the suspected material in a large test tube, and add 4 ml of concentrated hydrochloric acid. Prepare a spiral of 18-gage copper wire, approximately one-half inch in length, and clean by dipping it in concentrated nitric acid followed by thorough rinsing with water. Place the spiral in the test tube, and place the tube in a boiling water bath for at least 30 minutes. Mercury in appreciable quantity will impart a silvery sheen to the copper wire; presence of arsenic, antimony, or bismuth is indicated by a dark brown or black deposit on the copper. Since sulfides in the organic material being analyzed may cause the formation of a brown deposit on the copper, it is advisable to run confirmatory tests in all cases that are not definitely negative. Such tests may be found in any standard toxicology text (5).

## Discussion

It should be emphasized that in all cases the analyst should run a positive and a negative control with each analysis. The purity and stability of the reagents should be checked frequently. When, as is the case in the average routine laboratory, these analyses are run infrequently, it is advisable to prepare the reagents freshly each time. In no case should all the available material be used for the analysis. Blood or urine samples which have been sealed and frozen will keep indefinitely and will thus be available if any further analysis seems advisable.

## Summary

A series of tests has been presented for the detection and estimation of the more commonly occurring poisons which can be carried out in a routine biochemistry laboratory by an experienced technologist.

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# FIVE PARASITES INFECTING MAN AND METHODS FOR THEIR IDENTIFICATION*

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Parasites have been known since antiquity. The Egyptians knew about Ascari, Oxyuris, and Taenias fifteen centuries before the Christian era. In the Middle Ages, the study of parasitology was completely halted since no one dared to oppose the dogmas issued by Hippocrates and Galen. The discovery of the microscope in 1600 opened a new era in the history of parasitology, through the work of Leuwenhoeck, who, in 1683, first described bacteria.[1] However, due to the work of Pasteur and Davaine, bacteriology was much more thoroughly studied than parasitology. Finally, study and research developed parasitology into the complex field which it is now.

Parasitology has been more and more in use in the last few years, primarily due to the methods of transportation which have been developed. It has become so easy to travel south or north, or even from continent to continent, that parasites have been carried to regions in which they were never heard of before, sometimes imposing quite a problem in diagnosis.

*Endamoeba histolytica,* the first parasite under consideration, is the only pathogenic amoeba. Its primary location is in the mucosa of the cecum, sigmoid, and rectum. The trophozoite is the active, living form of this amoeba, and is found in liquid stools. The amoeba die quickly, so the stool should be examined as soon after collection as possible. The trophozoite under the microscope appears colorless, with a smooth endoplasm. The nucleus is not seen in the fresh specimen, and hyaline, tongue-like pseudopodia project rapidly. Ingested erythrocytes, if present, are diagnostic of *Endamoeba histolytica.* This pathogenic amoeba may be confused with the *Endamoeba coli,* which is harmless. *Endamoeba coli* only rarely will ingest erythrocytes, protrudes sluggish, granular pseudopodia, and has a visible nucleus.

The cystic form of *Endamoeba histolytica* is found in formed stools. In unstained fecal preparations, cysts appear as hyaline disks, similar to leukocytes. Identification of cysts depends on the internal morphology brought out by stains.

By using an iodine stain, it is possible to detect nuclei and chromatoidal bars. Chromatoidal bars are rod-shaped structures in the cytoplasm, which are diagnostic of *E. histolytica.* Both nuclei and the chromatoidal bars appear translucent against the darker staining of the organism itself. *E. histolytica* never has more than four nuclei, while *E. coli* may have as many as sixteen. Differences in sizes may be explained by the existence of large and small races of amoebae.

An iron-hematoxylin stain may be used as a further method of differentiation of *E. histolytica* from *E. coli.* This stain brings out the nuclear structure. *E. histolytica* shows uniform minute granules lining the inner wall of the nucleus, with a centrally located small karyosome. The chromatoidal bodies stain deeply. In *E. coli,* the chromatin lining the inner wall of the

[1] Gradwohl-Kouri, *Clinical Laboratory Methods and Diagnosis,* The C. V. Mosby Company, St. Louis, 1948, Vol. 111, 4th Edition, p. 1.

* Read by title, ASMT Silver Anniversary Convention, June, 1957.

nucleus is in masses and irregularly shaped; the karyosome is eccentrically located.

One other amoeba which may be confused with *E. histolytica* is the *Iodamoeba butschlii*. This amoeba, stained with iodine, shows a well defined glycogen mass with only one nucleus that takes a deep brown color.

The second parasite to be discussed is *Ascaris lumbricoides*, also known as the large intestinal roundworm. This parasite is prevalent in many areas of the United States but it is more common in the country than in the cities. The male and female worms live in the lumen of the small intestine, feeding on the intestinal contents. The female lays eggs, about 200,000 a day, which are passed in the feces. After the eggs are ingested in contaminated food or drink, the digestive juices act on fertilized eggs liberating the larvae, which penetrate the intestinal mucosa and are carried to the liver, then to the lungs, where they grow to 1.5 mm. long. When the larvae are coughed up and swallowed, they travel to the intestine and grow to adult worms.

The worms of *Ascaris lumbricoides* are large, and reddish yellow in color. The female is 20 cm. to 40 cm. long and about 0.5 cm. in diameter. The male is shorter than the female and has a curved tail. The eggs, viewed through a microscope, or oval, 45 m$\mu$ by 75 m$\mu$, with a transparent inner shell covered by a thick, irregular coat, which stains a deep brown color with iodine.

*Balantidium coli* is the third parasite which may be found in man. The original host is the pig; man becomes infected by the ingestion of cysts in contaminated food, or by the contaminated hands of individuals who slaughter hogs.

Examination of feces should be carried on as soon as possible after collection so that the living trophozoites can be seen. *Balantidium coli* is diagnosed readily because of its large size, the hair-like cilia covering its body, and by the large, kidney-shaped macronucleus. In some cases, the cytostome, or mouth opening may be seen.

The fourth parasite is the *Diphyllobothrium latum*, or fish tape-worm. Fresh-water fish are the intermediate hosts, so the parasite is found primarily in the lake districts of the world. In America the host fish are usually pike, pickerel, or wall-eyed pike. The adult form lives in the small intestine of fish-eating mammals. When the eggs reach fresh water, they are ingested by small crustacea, which, in turn, are eaten by other fish. When raw or poorly cooked fish is eaten by man, the young form of the tape-worm attaches itself to the small intestine and grows to adulthood in about six weeks. The adult worm may live twenty years and grow to a length of thirty feet.[1]

The head, or scolex, of the *Diphyllobothrium latum* is elongated, almond-shaped, and is provided with a dorsal and ventral sucker. The eggs, when seen with a miscroscope measure 80 $\mu$ by 40 $\mu$, oval-shaped, and are characteristically operculated.

The last parasite to be discussed is the *Trichinella spiralis*, which is a fairly common worm in America. The adults are found in the small intestine and are small, white, and just visible to the naked eye. The female is viviparous. The larvae are carried from the small blood vessel in the intestinal

[1] William G. Sawitz, M.D., *Medical Parasitology*, The Blakiston Company, Philadelphia, 1950. p. 116.

wall throughout the body. Eventually they become lodged in one of the tissues of the body. The larvae encysts, and finally becomes calcified in the skeletal muscle. Man acquires trichinosis by eating improperly cooked meat of animals that harbor the infection, the pig being the chief offender.

Although the worms are found in the intestine, they only rarely may be found in the feces. Larvae rarely may be found in the blood. Five ml. of blood is laked in 15 ml. of 3% acetic acid, the specimen is then centrifuged and the sediment examined.[1] Muscle biopsy is useful in diagnosis. The piece of muscle is pressed between two slides, and examined with the low power of a microscope. A skin test of powdered Trichinella larvae has also been found to be useful.

It is hoped that this discussion of five common parasites found in man will be of some value to the medical technologist.

[1] Sawitz, *Op. cit.*, p. 89.

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## ABSTRACTS

### SIMPLE METHOD FOR PROTECTING FRESH SMEARS FROM DRYING AND DETERIORATION DURING MAILING

George N. Papanicolaou and Emma L. Bridges (Papanicolaou Research Lab., Cornell University, Medical College, New York) J.A.M.A. 164, 1330-1 (1957).

The method presented in this report for mailing fresh, unstained smears has been used with gratifying results. The need for an adequate technique for mailing fresh unstained exfoliative cytology smears to the laboratory has been increasing.

Fresh, wet smears are covered immediately with a small quantity—approximately 0.25 or 0.5 cc. per smear—of Diaphane solution. This solution is 3 parts of 95% ethyl alcohol to 2 parts of Diaphane. After the Diaphane has dried thoroughly, in 20 to 30 minutes, a hard, smooth film protects the surface of the smear. The slides can then be wrapped in wax paper and mailed. When received in the laboratory, they are placed in alcohol-ether for approximately 20 minutes to remove the Diaphane; then they are stained according to the routine method for cytological smears.

The fixation, preservation, and staining of all types of cells in smears were found to be satisfactory after a lapse of two weeks. Another favorable point is that pouring of Diaphane over a glass slide held in a horizontal position results in a better spreading and an improved adhesiveness of the smear, which thus tends to be more cellular. If excess water is present in the specimen, the Diaphane film on the smear may appear milky and opaque instead of clear. Smears prepared from such specimens should, therefore, be adequately dehydrated in 95% alcohol for one or two minutes before the use of Diaphane.

### DETECTION OF MYELOMA PROTEIN IN URINE BY A NEW QUICK METHOD

P. Effersoe and B. Tidstrom (Kommunehospital, Copenhagen, Denmark) Jour. Lab. Clin. Med. 50, 134-8 (1957).

A method of detecting myeloma protein in urine is described, based on the differences in solubility at certain salt concentrations between myeloma protein and the protein occurring in urine from patients with nonmyelomatous renal diseases.

Twenty-one patients with myeloma proteinuria, the diagnosis in 18 had been made from the finding of myeloma cells in the bone marrow. Urine specimens from these 21 patients were submitted to a salting-out test as well as a temperature flocculation test. In the cases where one of the two tests were negative, electrophoretic analysis of the urinary protein was carried out also.

The salt used in this test: 3.6 molar equimolar mono- and dihydropotassium phosphate solution. The test is inapplicable for urine that cannot be filtered clear as well as for urine to which chemicals have been added, e.g. as bladder lavage. Readings can be taken immediately and must be taken within half an hour of mixing, because standing will also cause an inconstant increase of the turbidity. All readings were controlled by photometric determinations.

The salting-out test was positive in 19 of the 21 patients with myeloma proteinuria. In 7 cases with a positive salting-out test, but negative temperature flocculation test, the urinary protein was submitted to electrophoretic analysis which revealed myeloma protein in all cases. The temperature flocculation test was found positive in about 60% whereas the salting-out test was positive in about 90%.

# HOW TO PREPARE A TECHNICAL PAPER*

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## Introduction

At Armour Research Foundation, where I am employed, technical reports and papers are a very important phase of our operation. We do research under contract for government agencies and industrial organizations. The progress of the research on each project is periodically reported to the sponsor of that project. Each of the five largest departments at the Foundation has an editor or editors; one department has three. In addition, I review all the Foundation reports as a sort of "quality control" for technical writing.

Because we consider report writing so important, I also teach special report writing classes made up of engineers and scientists from the Foundation staff. This is a ten-step procedure that I give to the classes as a way to ease the labor of preparing reports and papers; you, too, may find it useful.

1. *Write and revise a bit at a time.*

Even for a professional writer, the hardest part of writing is getting started. There is something about a stack of blank paper and a typewriter or pen that causes some otherwise very competent people to become almost spastic. So, do not let everything go until the due date, but write item by item as your research progresses.

You can start writing your report the minute you decide upon your project. Start by writing down in your notebook the objectives of your project:

a. Why are you doing this research?
b. What do you hope to prove?
c. Why is this research necessary?

During the course of your research, you may find that you must limit, redefine, or discard some of your plans; such changes should be recorded and dated like any other notebook entry.

Write it in your notebook, not on scraps of paper that may be lost. One of the most valuable aids to writing a good technical report is keeping a good notebook, a sort of diary of your research life. During your training courses you were taught to record numerical data in your notebook or on special forms provided for the purpose. It is equally important to record such non-quantitative data as the peculiar color or odor of a specimen, that a reagent was accidentally contaminated, and possible causes of discrepancies in a certain set of determinations. Your notebook is a much better file of information than your memory.

Since "practice makes perfect" only when the correct act is practiced, take time to write complete sentences. Incomplete sentences are the most common characteristic of poor writing. Unfortunately, I have seen so many researchers puzzling over cryptic notations in their notebooks; they remind me of the story about Browning's being asked to interpret some lines in one of his poems. After puzzling over them, he finally

* Paper given at Silver Anniversary Convention of the American Society of Medical Technologists, June 26, 1957, Chicago, Illinois.
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confessed, "When I wrote those lines, only God and Browning knew what they meant. Now, only God knows." Six months after a notation was made is not the best time to decide whether, "Reaction occurs with heating", means that heat must be applied or that the reaction gives off heat.

In contemplating an idea for research, your first question is not just whether someone else had the same idea, but whether anyone has done anything about it. To answer that you start your research in the library. How to go about making a literature search is a very important subject, but we have time to discuss only those phases involved in writing a good technical paper. As you do your library reading, be sure to record for each article you read: the author, title, journal, volume, pages, and date. Be sure to list all the information needed for reference, not just to satisfy an editor but also to help you relocate the article if someone challenges your interpretation of another's work. Writing the notes on cards is preferable to recording such material in a notebook, because cards can be handled more easily both for locating information and for arranging the sequence and numbering of references for the bibliography. You may want to jot down only pertinent data from an article or you may want to abstract it. Sometimes you may want to copy sections for exact quotations.

Writing up your literature survey not only furnishes you with a background for your laboratory research, but also furnishes the introductory portion of your paper.

As you assemble your equipment and materials you can write up portions of your paper on equipment and materials. If only standard equipment, materials, and procedures are used, no description other than listing with identifying information is needed. However, any modifications of standard equipment, procedures, or materials should be described as should any new or special equipment, materials, and procedures.

Only modified and new equipment should be illustrated by photographs or drawings. Since photographic work and drafting take time, illustrations should be ordered as soon as the equipment is ready for operation. Because illustrations are costly, you should consult your organization style manual or journal requirements before ordering masters made.

2. *When you start writing your first complete draft, assemble all your source materials—your notebook, your literature search cards, and your illustrations.*

When the research project is finally completed, and you sit down to write your report, you will find that much of the report has already been written.

3. *Set up your tables and your graphs from the numerical data in your notebook.*

The reporting of data is the important distinction between technical writing and other expository writing. Much time is spent teaching technical students how to design graphs, and many books and articles have been written on the subject. Unfortunately, very little has been written on designing tables of data, so little is done about teaching tabulation principles, even in statistics classes.

The manner in which a table is set up may influence a reader's interpretation of the data reported, so great care should be exercised in deciding upon table formats. Tables of similar data should have similar formats.

Often a graph should be used instead of a mere tabulation of data because graphs can be used to show trends, comparisons, proportions, the effects of variables, etc.

4. *Determine and write out your conclusions.*

Tabulation and graphing of your data may point out important details that you may have overlooked while you were immersed in your research, busily looking for proof of your hypothesis. Also, it is very important that each conclusion be supported by substantial data. It is important that the report be written as the basis of the conclusions.

5. *Write the outline of your paper.*

If you are writing an organizational report, you probably have a style manual to guide you. In writing a paper for a journal you should organize your material according to journal requirements or custom. Probably the following outline would be satisfactory for an "American Journal of Medical Technology" article:

I. Introduction
   A. Explanation of the Problem
   B. History of Previous Research
   C. Justification for Article
II. Materials
III. Equipment
IV. Procedure
V. Experimental Results
VI. Conclusions and Discussion
VII. Acknowledgements
VIII. List of References.

However, the letter and numerical designations and the subtitles should not be used on the final draft sent to the editor.

6. *Start writing without worrying about grammar or spelling.*

Use your outline to get started, freewheeling, as it were. The outline is especially necessary if you are dictating your paper—either to a secretary or a machine. Often a man will say that he needs a secretary to take down his technical report because his thoughts race far ahead of his pen or his typing. I have known some men who dictate technical material efficiently to a machine, but only a very few who use secretaries effectively for such writing. In fact, I suspect that often the secretary is the real writer of such a researcher-secretary team; she may take down what the man says and then give back to him a report that retains just enough of his pet phrases to preserve his "delusions of grandeur."

But whether you scribble, type, or dictate, there comes a time when you must start to write that paper. Personally, I prefer to write longhand because I am a two-thumb typist. I seldom start at the beginning to write a technical report or paper because I find introductions so hard to write.

Quite often I start by rewriting and expanding the conclusions I wrote in Step 4 of this procedure. In the outline I suggested for an AJMT article, "Conclusions and Discussion" is Item VI. If I had five conclusions, I would list them as A, B, C, D, and E under VI. Then I would number any pages I wrote discussing the fourth conclusion as VI D-1, VI D-2, etc.; I would start the discussion of each conclusion

on a fresh page.

Often I pamper myself by getting off to a lazy start; I simply copy from my notebook those bits I have already worked out, such as the introduction; lists or descriptions of equipment, materials, and procedures; and lists of contributing personnel. As I write, I jump about from place to place in my outline; I start each section or major subsection on a fresh page and give it a page numbering sequence of its own. Sometimes I jot a "brilliant" idea on a sheet all by itself in the hopes that I shall be able to fit it in somewhere later. When I have covered all the titles and subtitles in my outline, I assemble the bits in order and renumber the pages in one sequence.

7. *Allow at least 12 hours to elapse, and then edit or rewrite your first draft.*

Most of the Foundation editors agree that convincing the men that they can improve their own writing is the biggest benefit of the report writing classes. Frequently during the first few sessions of a report writing class some one will insist that once he has written a report, he cannot take an objective attitude and criticize his own report. Often the last session of a report writing class turns into a regular Methodist prayer meeting with individuals testifying that a "cooling off" period, even just overnight, helps them review their own work objectively.

You should go over your first draft critically, checking it sentence by sentence for such factors as:

a. grammar
b. spelling
c. coherence
d. clarity
e. logic
f. parallel construction
g. conciseness.

Since most technical reports are by custom written in the third person, you probably should not use first and second person (I and you) as was used throughout this paper. Because technical writing necessarily contains long, technical words, you may improve the readability of your paper by trying to keep your sentences and paragraphs as short as possible. Remember that each paragraph should cover only one topic; try to write transitional sentences to lead from one paragraph to another.

Be sure to check your titles and subtitles for parallel construction; the easiest way to do this is to write out a complete table of contents. Tables of similar data should have similar formats, as should graphs of similar data.

8. *Write the abstract, if one is needed.*

The abstract should be the very last part of the report written. Usually, the abstract is the most difficult part to write because it must be very brief, yet contain at least:

a. the objectives of the research
b. the methods used
c. the conclusions reached
d. the recommendations made.

Sometimes an abstract is used as the introduction to a report or paper. A good abstract should be informative, not just a table of contents in sentence form.

9. *Give the revised and retyped draft to a supervisor or coworker, or both, for constructive criticism and technical review.*

Making this second typed draft (third draft, if you typed the original draft in Step 6) is necessary to achieve a good paper. Even a professional writer does not expect to get by with less than two typed drafts before the final; yet much poor technical writing results because many novices do not revise or rewrite.

Perhaps this is the most difficult of the ten steps because it is not easy to accept criticism and to profit from it. After a hard day at the office, I sometimes wonder if our authors are as protective of their real, flesh-and-blood children as they are of their reports, brain children. Many times criticism will be in the form of questions as to the meaning of certain portions; these should be rewritten.

10. *Prepare final copy of text, tables, illustrations and bibliography for submission to the editor.*

## Conclusion

Much more could be said about the task of preparing a technical paper; whole courses could be taught on it. These are just a few of the many pointers I could suggest to make the task easier. The only way to learn to write a technical paper is to write your first and to continue to write. In some respects it is like learning to drive a car. Though relatively few people earn their living by driving, almost all American adults can drive with varying degrees of skill. We would be amused by anyone who refused to learn to drive because he cannot become a professional at it, yet many research men and engineers expect to be excused from writing reports and papers because they are not professional writers.

I would like to recommend to you the American Medical Writers Association (209-224 W.C.U. Building, Quincy, Illinois), an organization of physicians, medical students, editors of medical journals, and others interested in improving medical writing. I have found their annual symposia on medical writing most interesting and helpful.

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# THE ABSTRACT OF THE TECHNICAL REPORT*

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My opinions on abstracts of technical reports are based chiefly on my five years of experience as a technical editor at Armour Research Foundation. The Foundation does research under contract for government agencies and industrial organizations, and the sponsor of each contract is kept informed of the progress of his research project by means of periodic technical reports. The Foundation usually has 500 current projects, so we process from 2000 to 3000 technical reports a year.

In general, two types of abstract section of technical reports are recognized: the descriptive and the informative. The descriptive abstract may be nothing more or less than the table of contents written in sentence form. An informative abstract will briefly state the problem, the method of conducting the investigation, the conclusions reached, and the recommendations made. The informative summary, of not more than one page, is the type preferred at Armour Research Foundation. A model ARF abstract contains a statement of the objective of the research and is a synopsis of the body of the report with emphasis on the results, conclusions, and recommendations.

Sometimes the abstract of a technical report may be referred to as a synopsis, a summary, or an epitome. However, even though an abstract may be called a summary, it should not be confused with the concluding summary sometimes placed at the end of a report. A concluding summary brings the report to a logical and obvious ending on the basis of the over-all discussion of results, conclusions, and recommendations.

There are two fundamental differences between an abstract and a summary:

(1) The abstract is written in narrative form, and the summary is written in expository or even argumentative form.

(2) The abstract is complete in itself while the summary depends upon the discussion in the report.

The abstract merely narrates the main points of a report. The summary explains the logic of the conclusions and the recommendations for future work. As such, the summary cannot be read out of context because it is dependent upon the rest of the report. It is written in a manner designed to convince or persuade the reader that the conclusions reached are the logical interpretation of the data.

In editing reports one can usually recognize the neophyte writer by his abstracts. Either he writes a purely descriptive abstract or he lifts whole sentences bodily from the report and repeats them in his abstract. Sometimes at the Foundation the descriptive abstract is the result of an effort to stay within the preferred one-page limit. Obviously it is almost impossible to write an informative one-page abstract of a report of 300 or more pages, so abstracts for very long reports are exceptions to the rule.

The other practice of the neophyte, copying sections verbatim from the report, is perhaps as good a way as any to write the *first draft* of an abstract. But succeeding revisions and drafts should be greater and greater condensations until the final draft is an abstract containing only one to five per cent as many words as the report, itself, certainly not more than 10 per cent. After all, too long an abstract defeats the purpose of writing one at all.

And that brings up a point that might have been used to start this discussion: What is the purpose of writing an abstract? Perhaps this can best be answered by considering who reads abstracts and why.

At the Foundation we say the abstract of a report is designed primarily to inform management, and we back this up by routinely sending only the abstract (without the report) to our top administration. Probably neither Foundation management nor a sponsor's management can take the time to read a 300-page final report, yet both want to know the results and the recommendations. Foundation management needs to be reminded of the objective of the research and, being scientific, wants to be informed briefly what methods were used to solve the problem.

* [Reprinted from Journal of Chemical Education, Vol. 32, Page 532, October, 1955.]
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[1] Presented before the Division of Chemical Literature at the 127th Meeting of the American Chemical Society, Cincinnati, March, 1955.

Actually, the Foundation type of abstract will also meet the needs of the busy scientist who skims the abstract to determine whether a report contains enough of interest in his research for him to take the time to read the entire report.

It seems to me that there is now a third and very important type of abstract reader who should be considered in writing a report abstract—the librarian. Fortunately, the traditional librarian, the custodian of books, is being replaced by a new type of librarian, one who recognizes that her job is not counting books but is getting them into the hands of those who need them. To indicate this trend from book storage to book referral there is an increasing use of the terms "information service" and "information specialist" (or information officer) to replace the words library and librarian. This trend is particularly apparent in the document or report libraries where the documents or reports are often unpublished in the sense that distribution may be restricted and that the total number of existing copies of any one report may be counted in the dozens rather than the thousands.

Although the information specialist probably has some scientific background, she cannot hope to understand all the reports that come to her for cataloguing and referral. It is obvious that if a report is catalogued under the wrong subject headings it may never be found by the people who need it. Actually there is less danger of wrong cataloguing from an abstract than of incomplete cataloguing. So it is important that an abstract should contain the pertinent information stressed in the report itself. As Jeanne North[2] said:

The abstract will become even more important if the trend toward catch-word cataloguing continues. In Dr. Mortimer Taube's system for library cataloguing, the subject headings to represent a report in the card catalogue are to be chosen from the important words noted in the abstract. This streamlined procedure puts the burden of adequately covering the subject matter and applications of a report squarely on the abstractor.

And that brings us to the question: Who should write the abstract? Everyone agrees that the abstract should be the very last part of the report written, but there are two schools of thought as to who should write it. Some recommend that the abstract should be written by someone other than the author of the report. Usually they recommend that the author's supervisor write the abstract so that he will make a more critical scientific evaluation of the report. In my own experience this results in a delay of anywhere from two days to three weeks, depending on the supervisor and often, alas, on how late the report is when it reaches him. It seems to me that the same goal can be achieved relatively painlessly by asking the supervisor to reread the abstract after he has read the report to determine whether:

(1) The report covers all the items mentioned in the abstract;

(2) The abstract and the report are in agreement as to the relative importance of items of information; for instance, the points stressed in the report are also stressed in the abstract;

(3) The abstract is as brief as feasible.

These three check points are adapted from some suggested by Gray.[3]

The abstract is actually the most difficult part of the report to write because it must cover all the salient points of the report concisely. The report writer is best prepared to write the abstract of his own report. He knows the novelty of his equipment or procedure, the most important data he reported, and the importance of his conclusions and recommendations. If the supervisor critically compares the report and the abstract according to the three check points given, the desired critical scientific evaluation of the report is achieved in one-tenth the time it would take him to write the abstract. After all, the abstract of the report is not a disciplinary exercise to make sure the supervisor does his job in the most time-consuming way possible. Certainly the writer may find it difficult to judge his report objectively when he writes his abstract, but the supervisor and even the editor can supply that objectivity when comparing the abstract with the report.

Of course, there is the compromise of having both the author and the supervisor write abstracts. The supervisor would then read the report and write his own abstract before reading the author's abstract. This compromise, like most compromises, is unsatisfactory. It does not save time, but rather requires more time.

[2] North, J. B., "Editing: stumbling block or stepping stone?" Paper presented before Technical Writers and Editors Session, American Documentation Institute, Cleveland, November 5, 1954.

[3] Gray, Dwight E., "How readable is your technical report?" J. Chem. Educ., 26, 374-6 (1949).

It, too, resembles schoolroom discipline. In addition, there is the problem of deciding between two pieces of writing, and no father can be more proud and protective of his offspring than an author can be of his writing.

In some respects, the technical report writer should follow the advice of the old-time preacher who said: "First I tell them what I am going to tell them, then I tell them, and then I tell them what I've told them," by using the abstract, the body of the report, and the summary for his three tellings.

## SOME FUNDAMENTALS OF DESIGNING TABLES OF DATA*[1]

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In exchanging shop talk with other report editors, I am always amused at the wide variety of tasks management sees fit to assign to the report editor; the job seems to be the catch-all for any unassigned duties. Yet I am sure that teaching has not been recognized as one of the basic duties of good technical editing. A writer should be able to learn something from the editing of his report, so that his next report will be better. If a writer's sixth report requires the same kind and amount of editing as his first, something is radically wrong. The blame probably can be placed upon unreasonable editorial requirements or upon poor author-editor relations.

Since poor spelling and grammar are the most common and most obvious weaknesses of technical writers, I suppose each of us has his own subtle methods of teaching these subjects to his writers.

But what do you do about teaching your men to report data intelligently? Since space is limited, I shall discuss only one phase of the problem, the design of tables of data. I am sure that on the problems of spelling and grammar all of us have been tempted to give up and just correct the mistakes without trying to improve our writers. But we cannot do that with tables of data because the scientist who collects the data is best equipped to interpret and evaluate them, and the design of a good table is dependent upon interpretation and evaluation.

So I have assembled a few fundamentals of tabulation which I have found useful in helping others design tables for the presentation of their data. These fundamentals can best be explained by examples, so I have "faked" some tables to illustrate my points. To borrow a technique from advertising, we might label almost all the A tables "Before" and the B ones, "After."

(1) *If four or more items of data are given, they should be presented in tabular form.*

In Example 1 notice how much easier it is to understand the table.

(2) *Every table should have both a number and a title.*

The title should be brief, but it should clearly identify the information contained, with reference to the text. If you follow the practice of listing table titles in the Table of Contents, your authors will realize that they cannot have a long list of identical titles but must individualize each.

(3) *Even qualitative comparisons and contrasts are more easily understood in table form.*

In Example 2, as in Example 1, reporting the data in a table is an aid to understanding.

Incidentally, it is important that your writers realize that not all data are numerical.

(4) *Columns and rows must have identifying headings.*

(5) *Units of measurement must be given.*

Usually, the column or row headings are the logical locations for units. Lack of such identification seems to be the most common failing of tables.

(6) *The same unit of measurement should be used for comparable properties or dimensions.*

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## EXAMPLE 1

The daily low-X diet, called Diet A, was prepared by adding to 10 g. of skim milk the equivalent of 0.0003 g. of iron from $FeSO_4 7H_2O$, 0.0001 g. of zinc from $Zn(C_2H_3O_2)_2$, 0.0001 g. of copper from $CuSO_4 5H_2O$, 0.0026 mg. of iodine from KI, and 0.0008 g. of manganese from $MnSO_4 H_2O$.

**TABLE 1**

**Low-X Control Diet, Diet A**

| MINERAL | Mg./10 g. Skim Milk | Source |
|---|---|---|
| Fe | 0.3 | $FeSO_4 7H_2O$ |
| Zn | 0.1 | $Zn(C_2H_3O_2)_2$ |
| Cu | 0.1 | $CuSO_4 5H_2O$ |
| I | 0.0026 | KI |
| Mn | 0.8 | $MnSO_4 H_2O$ |

## EXAMPLE 2

Streaked animals, ss, can be produced only by the following matings: (*a*) streaked ss × streaked ss, (*b*) heterozygous Ss × streaked ss, (*c*) heterozygous Ss × heterozygous Ss. In mating (*a*) all offspring will be streaked; in mating (*b*) in which one of the parents was streaked ss and the other heterozygous Ss, half of the offspring will be streaked; in mating (*c*) in which both parents are heterozygous, one-fourth of the offspring will be streaked.

**TABLE 2**

**Matings Required to Produce Streaked Animals, ss**

| PARENTS | Streaked Offspring, ss |
|---|---|
| Streaked ss × streaked ss | All |
| Heterozygous Ss × streaked ss | ½ |
| Heterozygous Ss × heterozygous Ss | ¼ |

## EXAMPLE 3

**TABLE 3A**

| Test | Sample | Duration, Min. | Ash Type | Velocity, f.p.s. | Concn., gr./ft.³ | Ash impinged, g. | Ash Collected, g. | Count Rate, c.p.m. | Steel Collected, g. | Steel Expected, g. |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 10 | .. | 1050 | .. | .... | ..... | 33 | ..... | ..... |
| 2 | 2 | 10 | .. | 1050 | .. | .... | ..... | 76 | ..... | ..... |
| 3 | 1 | 10 | A | 1050 | 1.3 | 1.0 | 0.71 | 2523 | 0.024 | 0.04 |
| 4 | 1 | 10 | A | 1050 | 1.3 | 1.0 | 0.54 | 1446 | 0.014 | 0.04 |
| 5 | 1 | 10 | A | 1050 | 1.3 | 1.0 | 0.58 | 1479 | 0.014 | 0.04 |
| 6 | 2 | 10 | A | 1050 | 1.3 | 1.0 | 0.54 | 2227 | 0.021 | 0.04 |
| 7 | 2 | 10 | A | 1050 | 1.3 | 1.0 | 0.53 | 2261 | 0.021 | 0.04 |
| 8 | 2 | 10 | B | 1050 | 1.3 | 1.0 | 0.23 | 473 | 0.004 | 0.015 |
| 9 | 2 | 10 | B | 1050 | 1.3 | 1.0 | 0.17 | 423 | 0.004 | 0.015 |
| 10 | 2 | 10 | B | 1050 | 1.3 | 1.0 | 0.16 | 502 | 0.005 | 0.015 |
| 11 | 2 | 10 | B | 800 | 1.6 | 1.0 | 0.23 | 395 | 0.004 | ... |
| 12 | 2 | 10 | B | 800 | 1.6 | 1.0 | 0.26 | 331 | 0.003 | ... |
| 13 | 2 | 10 | B | 800 | 1.6 | 1.0 | 0.28 | 376 | 0.003 | ... |
| 14 | 2 | 10 | A | 800 | 1.6 | 1.0 | 0.92 | 700 | 0.006 | ... |
| 15 | 2 | 10 | A | 800 | 1.6 | 1.0 | 1.00 | 629 | 0.006 | ... |

**Note:** Fly ash type A, untreated; Fly ash type B, Aerotec separated. Sample 1, flat surface; 2, curved surface.

Notice in Table 1 of Example 1, that all the weights are converted to milligrams.

(7) *The units of measurement used in a table should all be of the same system, such as metric, English, or apothecary.*

Of course, your engineers will insist that their convention demands a mixture.

You may need to ask them to cite examples from printed literature to justify their usage.

(8) *If an item is repeated several times in a table, probably it should appear just once in the title, in a footnote, or in a column or row head.*

Often such repetition obscures important similarities, as you can see by inspection of the two tables in Example 3.

The third and seventh columns of Table 3A have been eliminated in Table 3B by inserting the information in the title. The fourth, fifth, and eleventh columns of Table 3A have been eliminated in Table 3B, and the use of the information in inserts serves to group related data.

See Example 4, also.

(9) *Column and row headings should be used to group related data.*

See the B tables of Examples 3 and 4.

**TABLE 3B**

**Erosion Data for 10-Minute Impingement of 1-g. High-speed Fly Ash on a Radioactive Steel Alloy**

| Steel Surface[a] | Test | Ash Recovered, g. | Counting Rate, c./min. | Collected, g. |
|---|---|---|---|---|
| 2 | 1 | .... | 33 | .... |
| | 2 | .... | 76 | .... |
| | **1.3 gr./ft.³, ash A[b] at 1050 ft./sec.** | | | |
| 1 | 3 | 0.71 | 2253 | 0.024[c] |
| | 4 | 0.54 | 1446 | 0.014 |
| | 5 | 0.58 | 1479 | 0.014 |
| 2 | 6 | 0.54 | 2227 | 0.021 |
| | 7 | 0.53 | 2261 | 0.021 |
| | **1.3 gr./ft.³, ash B[b] at 1050 ft./sec.** | | | |
| 2 | 8 | 0.23 | 473 | 0.004[d] |
| | 9 | 0.17 | 423 | 0.004 |
| | 10 | 0.16 | 502 | 0.005 |
| | **1.6 gr./ft.³, ash B[b] at 800 ft./sec.** | | | |
| 2 | 11 | 0.23 | 395 | 0.004 |
| | 12 | 0.26 | 331 | 0.003 |
| | 13 | 0.28 | 376 | 0.003 |
| | **1.6 gr./ft.³, ash A[b] at 800 ft./sec.** | | | |
| 2 | 14 | 0.92 | 700 | 0.006 |
| | 15 | 1.00 | 629 | 0.006 |

[a] 1, flat; 2, curved. [b] A, untreated; B, Aerotec separated.
[c] 0.04 g. expected. [d] 0.015 g. expected.

**TABLE 4A**

**Summary of Ozonolysis Runs**

| Ozonolysis Run No. | Ozone Concentration, Carrier Gas, Solvent, and Temperature | Critical Variables in Processing | Yield, Percent |
|---|---|---|---|
| 69 | 10% $O_3/N_2$ in 2.83 g. X, 32 ml. HAc, 20-25° C. | Heated 2 hr. at 90-95° C.; half charge (5%) $O_3$ passed through at 25° C. over 15-min. period | Charred |
| 70 | 5% $O_3/N_2$ in 0.85 g. X, $CCl_4$/HAc; 31/8 ml., 23-24° C. | Allowed to stand 3 days at 25° C.; added 15 ml. HAc; refluxed 0.5 hr. | 80 |
| 71 | 10% $O_3/N_2$ in 2.83 g. X, 32 ml. $CCl_4$, 21-24° C. | Added 30 ml. HAc; heated 2 hr. at 80-95° C.; 1 ml. $H_2O$ added; half charge $O_3$ added during 30 min. at 25° C. | 40 |
| 72 | 5% $O_3/N_2$ in 0.85 g. X, $CCl_4$/HAc; 31/8 ml., 23-24° C. | Held 2 hr. at 25° C.; added 15 ml. HAc; refluxed 0.5 hr. | 77 |

**TABLE 4B**
**Summary of Ozonolysis Runs 69 to 72**

| Run No. | OZONIZATION | | | | DECOMPOSITION | | | | Yield, %[a] |
|---|---|---|---|---|---|---|---|---|---|
| | $O_3/N_2$, % | X, g. | Solvent, ml. | Temp., °C. | $O_3/N_2$, % | Treatment | Time, Hr. | Temp., °C. | |
| 69 | 10 | 2.83 | HAc 32 | 20–25 | 5 | Heated | 2 | 90–95 | Charred |
| | | | | | | $O_2$ added | ¼ | 25 | |
| 70 | 5 | 0.85 | $CCl_4$ 31 | 23–24 | | Held | 72 | 25 | 80 |
| | | | HAc 8 | | | 15 ml. HAc added | | | |
| | | | | | | Refluxed | ½ | | |
| 71 | 10 | 2.83 | $CCl_4$ 32 | 21–24 | 5 | 30 ml. HAc added | | | 40 |
| | | | | | | Heated | 2 | 80–95 | |
| | | | | | | 1 ml. $H_2O$ added | | | |
| | | | | | | $O_2$ added | ¼ | 25 | |
| 72 | 5 | 0.85 | $CCl_4$ 31 | 23–24 | | Held | 2 | 25 | 77 |
| | | | HAc 8 | | | 15 ml. HAc added | | | |
| | | | | | | Refluxed | ½ | | |

[a] Based on amount of X used.

## EXAMPLE 5

**TABLE 5A**
**Some Physical Constants of the Brominated Hydrocarbons Used**

| CHEMICAL | $CBr_4$ | $C_2H_4Br_2$ | $CH_2Br_2$ | $C_2Br_4$ |
|---|---|---|---|---|
| Specific gravity | 3.42 | 2.055 | 2.495 | .... |
| Boiling point, °C. | 189.5 | 108 | 98.5 | 226 |

**TABLE 5B**
**Some Physical Constants of the Chlorinated Hydrocarbons Used**

| CHEMICAL | Specific Gravity | Boiling Point, °C. |
|---|---|---|
| $CCl_4$ | 1.59 | −22.6 |
| $CHCl_3$ | 1.50 | 62.0 |
| $C_2HCl_3$ | 1.465 | 87.2 |
| $C_2Cl_4$ | 1.63 | 120.8 |

## EXAMPLE 6

**TABLE 6A**
**Effect of Filter in Gas Line**

| TIME | Meter Readings, $Ft.^3$ | Flow Rate, c.f.m. | Temp., °F. | ΔP Across Trap in Hg |
|---|---|---|---|---|
| 10:00 | 66,830 | .. | 74 | 5.2 |
| 10:30 | 67,792 | 31 | 70 | 5.2 |
| 11:00 | 69,031 | 40 | 71 | 5.3 |
| 11:30 | 70,210 | 31 | 72 | 5.7 |
| 12:00 | 71,172 | 40 | 72 | 6.2 |
| 12:30 | 72,381 | 31 | 73 | 6.8 |

**TABLE 6B**
**Effect of Filter in Gas Line**

| 30-Min. Intervals | Flow Rate, $Ft.^3$/Min. | Temp., °F. | ΔP Across Trap in Hg |
|---|---|---|---|
| 1 | .. | 74 | 5.2 |
| 2 | 31 | 70 | 5.2 |
| 3 | 40 | 71 | 5.3 |
| 4 | 31 | 72 | 5.7 |
| 5 | 40 | 72 | 6.2 |
| 6 | 31 | 73 | 6.8 |

## EXAMPLE 7

Diet B included Diet A plus 20 micrograms of X (from $XCO_3$) per 10 g. of skim milk. Diet C was Diet A enriched with 20 micrograms of X (from $X_2O_3$) per 10 g. of skim milk.

**TABLE 7A**

**Results of Feeding Rats Low-X Diet A[a] for 8 Weeks**

| RAT | Weight, g. | | |
|---|---|---|---|
| | Starting | Final | Gain |
| $A_1$ | 70 | 135 | 55 |
| $A_2$ | 100 | 168 | 68 |
| $A_3$ | 80 | 186 | 106 |
| $A_4$ | 100 | 165 | 65 |
| $A_5$ | 130 | 202 | 72 |
| $A_6$ | 75 | 117 | 42 |
| $A_7$ | 110 | 150 | 40 |
| $A_8$ | 80 | 152 | 72 |
| $A_9$ | 85 | 135 | 50 |
| $A_{10}$ | 90 | 148 | 58 |
| Average | .. | .. | 63.8 |

[a] See Table 1.

**TABLE 7B**

**Comparison of Rat Weight Gains on Three X Test Diets[a] for 8 Weeks**

| DIET | X Added to Diet A[b] | | Average Gain, mg. |
|---|---|---|---|
| | g./10 g. Milk | Source | |
| A[b] | .. | .. | 63.8 |
| B | 20 | $XCO_3$ | 75.15 |
| C | 20 | $X_2O_3$ | 75.3 |

[a] Each diet was fed to a different group of 10 rats.
[b] See Table 1.

(10) *Similar tables should be set up in the same manner within a given report.*

If the data of both tables in Example 5 are to appear in the same report, they should be set up in the same manner, preferably like Table 5B.

(11) *Whenever possible, a table should be set up so that it can be typed on the page in the normal manner.*

See Example 3.

(12) *For ease of comparison, a table should be set up so that all the data in a given column have the same unit. If the items in a column have different units, each item should be centered in the column instead of aligned in the usual manner by digits or the decimal point.*

See Example 5.

(13) *Data of only record interest should not be included in a report table.*

The clock times listed in Table 6A of Example 6 are not pertinent to the problem. In fact, listing them obscures the pertinent fact that the readings were made at 30-min. intervals.

(14) *Often a summary table should be presented in the report and the supporting or record tables may be placed in an appendix.*

In example 7, Table 7B is a summary of the paragraph, of Table 7A, and of two similar tables giving the results of feeding diets B and C. Table 7A and the other two tables may be placed in an appendix to the report. Of course, such decisions are dependent upon the purpose of the report.

## ADDENDUM

15. *In a table reporting a series of experiments or tests with some factors constant for certain groups and other factors varying, the data should be grouped according to the constant factors when possible.*
16. *Usually the chronological order in which data were obtained is of little importance and the sequence of the data in the table should be determined by more significant factors.*
17. *Each type of data to be used in a table should be reported in a manner that will facilitate interpretation of all the data in the table.*